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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

MARINE BIOLOGICAL LABORATORY
WOODS HOLE
LIBRARY
MASS.

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JULY TO DECEMBER, 1927

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

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THIRTY-NINTH YEAR.

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I. TRUSTEES.

EX OFFICIO.

FRANK R. LILLIE, *President of the Corporation,* The University of Chicago.
MERKEL H. JACOBS, *Director,* University of Pennsylvania.
LAWRASON RIGGS, JR., *Treasurer,* 25 Broad Street, New York City.
L. L. WOODRUFF, *Clerk of the Corporation,* and *Secretary of the Board of Trustees pro tem,* Yale University.

EMERITUS.

CORNELIA M. CLAPP, Mount Holyoke College.
GILMAN A. DREW, Eagle Lake, Florida.

TO SERVE UNTIL 1930.

E. G. CONKLIN, Princeton University.
OTTO C. GLASER, Amherst College.

Ross G. Harrison, Yale University.
H. S. Jennings, Johns Hopkins University.
F. P. Knowlton, Syracuse University.
M. M. Metcalf, Johns Hopkins University.
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TO SERVE UNTIL 1929.

C. R. Crane, New York City.
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
E. P. Lyon, University of Minnesota.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, Columbia University.
D. H. Tennent, Bryn Mawr College.
E. B. Wilson, Columbia University.

TO SERVE UNTIL 1928

H. H. Donaldson, Wistar Institute of Anatomy and Biology.
W. E. Garrey, Vanderbilt University Medical School.
Caswell Grave, Washington University.
M. J. Greenman, Wistar Institute of Anatomy and Biology.
R. A. Harper, Columbia University.
A. P. Mathews, The University of Cincinnati.
G. H. Parker, Harvard University.
C. R. Stockard, Cornell University Medical College.

TO SERVE UNTIL 1927

H. C. Bumpus, Brown University.
H. E. Crampton, Barnard College.
W. C. Curtis, University of Missouri.
George T. Moore, Missouri Botanical Garden, St. Louis.
W. J. V. Osterhout, Member of the Rockefeller Institute for Medical Research.
J. R. Schramm, University of Pennsylvania.
William M. Wheeler, Bussey Institution, Harvard University.
Lorande L. Woodruff, Yale University.

EXECUTIVE COMMITTEE OF THE BOARD OF TRUSTEES.

Frank R. Lillie, *Ex. Off. Chairman.*
Merkel H. Jacobs, *Ex. Off.*
Lawrason Riggs, Jr., *Ex. Off.*
I. F. Lewis, to serve until 1927.
T. H. Morgan, to serve until 1927.
Otto C. Glaser, to serve until 1928.
Caswell Grave, to serve until 1928.

THE LIBRARY COMMITTEE.

C. E. McCLUNG, *Chairman.*
M. M. METCALF.
J. R. SCHRAMM.
E. E. JUST.
ROBERT A. BUDINGTON.
CHARLES J. FISH.
A. H. STURTEVANT.
ALFRED C. REDFIELD.
FRANK R. LILLIE.

II. ACT OF INCORPORATION.

No. 3170

COMMONWEALTH OF MASSACHUSETTS.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

[SEAL] HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio,* members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers sholl hold their respective offices until others are chosen and qualified in their stead. The President of the Corporation, the Director and the Associate Director of the Laboratory, shall also be Trustees, *ex officio.*

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER.

To the Trustees of Marine Biological Laboratory.

Gentlemen: As Treasurer of Marine Biological Laboratory, I herewith submit my report for the year 1926.

The books have been audited by Messrs. Seamans, Stetson & Tuttle, Certified Public Accountants (Successors to Messrs. Harvey S. Chase & Company, who for a number of years have audited the books). A copy of their report is on file at the Laboratory and is open to inspection by any member of the Corporation.

The principal of the Endowment Fund at the close of the year consisted of securities of the book value of $906,337.50 and cash $112.00. The Income from the Endowment Fund was $47,517.48, and the fee of the Trust Company as Trustee was $787.50, leaving a net income from the Endowment Fund of $46,729.98. A full list of the securities will be found in the Auditor's report.

There was no change in the securities of the Lucretia Crocker Fund during the year. The cash in this Fund at the end of the year was $587.77.

The land, buildings, library and the equipment of the Laboratory excluding the Gansett and Devil's Lane property and the buildings under construction, represents an investment of $1,263,343.05, and after deducting $107,444.95 for depreciation, a net book value of $1,155,898.10.

During the year $100,000 was received from the General Education Board on account of their appropriation of not to exceed $250,000 for the construction of the new apartment house and dormitory, out of which receipt $47,889.86 was paid on account to contractors, leaving a cash balance of $52,247.71.

The Retirement Fund was set up at the end of 1925 consisting of 5 per cent. of the permanent pay roll, to which additions are made at the end of each year on this basis. At the end of 1926 the Fund consisted of:

Securities	$2,200.00	
Cash	2,443.58	$4,643.58

most of the cash being the appropriation for the year 1926, which has been subsequently invested.

During the year the entire balance of the Gansett mortgage, and $5,000 on account of the Drew mortgage, and $1,000 on account of the Danchakoff mortgage, were paid off, and the indebtedness of the Laboratory at the end of the year consisted of $5,573.25 in Accounts Payable, and $44,500 in mortgages on its real estate.

The actual receipts and disbursements closely approximated the estimated figures and the expenditures were within the income.

Following is the balance sheet at the end of the year and the condensed statement of income and outgo for the year as reported by the Auditors.

EXHIBIT A.

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1926.

Assets

Endowment Fund Assets:			
Securities in Hands of Trustee—Schedule I		$ 906,337.50	
Investment Cash in Hands of Trustee		112.00	
		$ 906,449.50	
Lucretia Crocker Fund Assets,			
Securities—Schedule II	$ 4,011.17		
Cash—Schedule II	587.77	4,598.94	$ 911,048.44

MARINE BIOLOGICAL LABORATORY LIBRARY WOODS HOLE, MASS.

Plant Assets:			
Land—Schedule III	$113,167.55		
Buildings—Schedule III	958,975.88		
Equipment—Schedule III	121,885.17		
Library—Schedule III	69,314.45	$1,263,343.05	
Less Reserve for Depreciation		107,444.95	
		$1,155,898.10	
New Dormitory Buildings Construction Account	$ 47,889.86		
Cash in Dormitory Buildings Fund Account	52,247.71	100,137.57	1,256,035.67
Current Assets:			
Cash,			
In New York Bank	$ 522.15		
In Hands of Trustees	3,575.00		
In Falmouth Bank	1,562.59		
Petty Cash	500.00	$ 6,159.74	
Accounts—Receivable		$ 20,081.98	
Inventories,			
Supply Department	$ 25,865.03		
Biological Bulletin	5,881.12	31,746.15	
Investments,			
Devil's Lane Property	$ 31,919.46		
Gansett Property	2,530.88		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	4,643.58	51.793.92	
Prepaid Insurance		4,353.89	114,135.68
			$2,281,219.79

Liabilities.

Endowment Funds:			
Friendship Fund, Inc.		$ 405,000.00	
John D. Rockefeller, Jr.		400,000.00	
Carnegie Corporation		100,000.00	
Gain on Sale of Securities		1,449.50	
		$ 906,449.50	
Lucretia Crocker Fund		4,598.94	$ 911,048.44

Plant Funds:		
Rockefeller Foundation	$ 500,000.00	
Friendship Fund Gift of 1925	221,608.61	
General Education Board	100,000.00	
Other Investments in Plant from Gifts and from Current Funds	414,789.49	
	$1,236,398.10	
Real Estate Mortgages on Drew and Danchakoff Properties	19,500.00	
Suspense—Interest on Building Fund Cash	137.57	1,256,035.67
Current Liabilities and Surplus:		
Mortgage Note on Devil's Lane Property	$ 25,000.00	
Accounts—Payable	5,573.25	
Items in Suspense	58.79	
	$ 30,632.04	
Current Surplus—Exhibit C	83,503.64	
		114,135.68
		$2,281,219.79

EXHIBIT B.

MARINE BIOLOGICAL LABORATORY, INCOME AND EXPENSE FOR THE YEAR ENDED DECEMBER 31, 1926.

	Total.		Net.	
	Expense.	Income.	Expense.	Income.
Income, Endowment Fund		$ 47,517.48		$47,517.48
Donation from General Education Board for Purchase of Books		10,000.00		10,000.00
Instruction	$ 7,556.63	10,475.00		2,918.37
Research	3,125.42	11,650.00		8,524.58
Biological Bulletin and Membership Dues	5,876.06	7,171.54		1,295.48
Supply Department, Schedule IV	56,539.67	58,213.60		1,673.93
Mess, Schedule V	29,319.52	33,547.55		4,228.03
Dormitories, Schedule VI	7,927.96	5,680.60	$ 2,247.36	
Interest and Depreciation charged to above three Departments. See Schedules IV, V, and VI (red)	14,695.41			14,695.41

Dividends on Stock in General Biological Supply House, Inc.		2,032.00		2,032.00
Rent of Danchakoff Cottage	226.26	700.00		473.74
Rent of Microscopes		312.00		312.00
Sales of Junk		264.25		264.25
Interest on Bank Balances		119.22		119.22
Newman Cottage	78.93	150.00		71.07
Rent of Garage, Railway, etc.		101.47		101.47
Sundry Income		44.40		44.40
Maintenance of Plant:				
New Laboratory Expense	15,102.30		15,102.30	
Maintenance of Buildings and Grounds	9,429.95		9,429.95	
Chemical and Special Apparatus Department	6,782.92		6,782.92	
Library Department Expense	7,354.75		7,354.75	
Carpenter Department Expense	1,471.10		1,471.10	
Truck Expense	1,000.29		1,000.29	
Sundry Expenses	964.67		964.67	
Bar Neck Property Expense	375.00		375.00	
Evening Lectures	210.32		210.32	
General Expenses:				
Administration Expenses	11,648.33		11,648.33	
Interest on Loans	1,380.00		1,380.00	
Endowment Fund Trustees	787.50		787.50	
Bad Debts	207.55		207.55	
Reserve for Depreciation	27,513.08		27,513.08	
		$187,979.11		$94,271.43
	$180,182.80	180,182.80	$86,475.12	86,475.12
Balance of Income carried to Current Surplus—Exhibit C		$ 7,796.31		$ 7,796.31

Respectfully submitted,

LAWRASON RIGGS, JR.,

Treasurer.

V. THE REPORT OF THE LIBRARIAN, DECEMBER 31, 1926.

The year's acquisitions and accomplishments are greater than those of any year in the history of the library except perhaps of the year when the American Museum turned over to our library many valuable back sets of serials on a long-time loan. This year. the advance over the past ten is in direct proportion to the increase in our available funds due to the generous gift from the General Education Board of $10,000 to be spent in the purchase of back sets and of expensive reference works and monographs. While the $10,000 was spent for the precise purpose to which it was designed, a sum from our own annual budget that we should have spent toward this purpose had we been dependent upon our budget alone, was thus released to furnish extra help, not only in connection with the purchases made possible by this sum from the General Education Board, but also in carrying out certain accumulated tasks that have now by the end of the year been practically accomplished.

Of serials there were 41 back sets completed, the most notable of these sets being the *Comptes Rendus* of the French Academy, *Virchow's Archiv,* and *Die Naturwissenschaften.* There were 33 back sets nearly completed, the most notable of these being *Pflüger's Archiv.* By exchange we secured 5 complete back sets and 9 partially complete back sets.

One hundred and eleven serials were added to our current receipts, 30 by subscription and 81 by exchange. Many will recall the lists of possible serials that were submitted to the investigators during the summer of 1925. From among those for which we received requests we secured 111 newly undertaken subscriptions and exchanges.

There were several monographs and reference books purchased among which were Retzius' "Biologische Untersuchungen," Richet's "Dictionnaire de Physiologie," "Norwegian North-Atlantic Expedition," and Glazenbrook's "Dictionary of Physics." Abderhalden's "Handbuch" is still being purchased as it comes from the press. Two hundred and eighty-seven books, not expensive reference works or monographs, were added to the library, 133 by purchase and 154 by gift.

Many publishers in this country have now made acknowledgment of the value to them of coöperation with this library. The effort to secure evidence of the fact that men order books for their college libraries and for their own libraries, from having seen them here, will continue and this evidence will then be sent on to the publisher. We wish to call the very especial attention of the investigators to this point. Concrete proof that the presence of books on display in our " new books " corner of the reading room, does lead to sales, is the only evidence that will induce publishers to continue and to increase these gifts. During the summer of 1926 twelve persons made due acknowledgment and 40 books examined here were recorded as being ordered.

The list of books presented, with the names of the donors in full, follows except for certain instances where we were specifically requested not to publish the information.

From the publisher, Félix Alcan: Petronievics, Branislav: *L'Evolution Universelle.*

E. Arnold and Co.: Cochrane, J. A.: *A School History of Science.*

G. Bell and Sons, Ltd.: Dell, J. A.: *Animals in the Making.*

P. Blakiston's Son and Co.: Brubaker, Albert P.: *Text-book of Human Physiology*; Curtis, Francis D.: *A Digest of Investigations in the Teaching of Science*; Frank, J. O.: *How to Teach General Science*; Jackson, C. M.: *The Effects of Inanition and Malnutrition upon Growth and Structure*; Jackson, C. M.: *Morris' Human Anatomy*; Kingsley, J. S.: *The Vertebrate Skeleton, from the Developmental Standpoint*; Patten, Bradley M.: *Early Embryology of the Chick*; Strecker, Edward A. and Ebaugh, F. G.: *Practical Clinical Psychiatry for Students and Practitioners*; and Whetham, Wm. Cecil D.: *The Recent Development of Physical Science.*

Chemical Catalogue Co.: Kraus, Charles A.: *The Properties of Electrically Conducting Systems, including Electrolytes and Metals.*

Wm. T. Comstock and Co.: Riley, Wm. A. and Johannsen, O. A.: *Handbook of Medical Entomology.*

Thos. Y. Crowell Co.: Eldridge, Seba: *Organization of Life.*

E. P. Dutton & Co.: Jennings, H. S.: *Prometheus.*

Ginn & Co.: Taylor, Lloyd Wm.: *College Manual of Optics*; and Ward, Robert De Courcy: *Climates of the United States.*

Harcourt, Brace & Co.: de Kruif, Paul: *Microbe Hunters*; Holmes, S. J.: *Life and Evolution*; and Holmes, S. J.: *Trend of the Race.*

Harvard University Press: Do Amaral, Afranio: *South American Pit-vipers*; Castle, W. E.: *Genetics and Eugenics*; Parker, G. H.: *What Evolution Is*; and Williston, Samuel W.: *The Osteology of the Reptiles.*

W. Heffer & Sons, Ltd.: Grey, Egerton Charles: *Practical Chemistry for Micro-methods*; and Michaelis, Leonor: *Practical, Physical and Colloid Chemistry.*

Norman W. Henley and Co.: Jacobs, Frederic Burnham: *Abrasives.*

Henry Holt and Co.: Osterhaut, W. J. V.: *Nature of Life*; and Wilder, Harris H.: *The Pedigree of the Human Race.*

Houghton Mifflin Co.: Barbour, Thomas: *Reptiles and Amphibians, their Habits and Adaptations.*

Alfred A. Knopf: Pearl, Raymond: *The Biology of Population Growth*; and Singer, Charles: *The Evolution of Anatomy.*

Lea and Febiger: Appleton, J. L. T., Jr.: *Bacterial Infection with Special Reference to Dental Practice*; Bush, Arthur Dermont: *A College Text-book of Physiology*; Calkins, Gary N.: *Biology of the Protozoa*; Collins, E. Treacher: *Arboreal Life and the Evolution of the Human Eye*; Cummer, Clyde Lottridge: *A Manual of Clinical Laboratory Methods*; Greaves, Joseph E.: *Agricultural Bacteriology*; Hargitt, Charles W. and Hargitt, Geo. T.: *Outlines of General Biology*; Park, Wm. H., Williams, A. W. and Krumwiede, Chas.: *Pathogenic Microörganisms*; Phillips, Ruth L.: *Vertebrate Embryology*; Richards, A.: *A Laboratory Guide in General Zoology*; Roussy, Gustav and Bertrand, Ivan: *Lessons in Pathological Histology*; and Smallwood, Wm. Martin: *A Text-book of Biology for Students in General, Medical and and Technical Courses.*

H. K. Lewis and Co.: Bond, C. J.: *The Leucocyte in Health and Disease.*

J. B. Lippincott Co.: Villager, Emil: *Brain and Spinal Cord.*

McGraw-Hill Book Co.: Eames and MacDaniels: *An Introduc-*

tion to Plant Anatomy; Eucken, Arnold: *Fundamentals of Physical Chemistry*; Mitchell, Philip H.: *A Textbook of General Physiology for Colleges*; Sharp, Lester W.: *An Introduction to Cytology*; Sinnott and Dunn: *Principles of Genetics*; Snodgrass, R. E.: *Anatomy and Physiology of the Honeybee*; and Wieman, H. L.: *General Zoology.*

The Macmillan Co.: Brinkley, Stuart R.: *Principles of General Chemistry*; Hegner and Taliaferro: *Human Protozoology*; Hogben, L. T.: *Comparative Physiology*; Kyle, Harry M.: *The Biology of Fishes*; O'Toole, G. B.: *Case against Evolution*; Shipley, Sir Arthur E.: *Life*; Thurstone, L. L.: *The Fundamentals of Statistics*; and Whitehead, Alfred N.: *Science and the Modern World.*

Marshall Jones Co.: Loomis, Frederic Brewster: *The Evolution of the Horse.*

Masson et Cie: Calmette, A., Nègre, L. and Boquet, A.: *Manuel Technique de Microbiologie et Sérologie.*

Oliver and Boyd: Fisher, R. A.: *Statistical Methods for Research Workers*; and Ponder, Eric: *The Erythrocyte and the Action of Simple Haemolysins.*

Open Court Publishing Co.: Bliss, Gilbert Ames: *Calculus of Variations*; Cassirer, Ernst: *Substance and Function and Einstein's Theory of Relativity*; Curtiss, David Raymond: *Analytic Functions of a Complex Variable*; and Sellars, Roy Wood: *Evolutionary Naturalism.*

Oxford University Press: Joly, J.: *Radioactivity and the Surface History of the Earth*; and Wissler, Clark: *The Relation of Nature to Man in Aboriginal America.*

Premier Publishing Co.: Meisel, Max: *A Bibliography of American Natural History, the Pioneer Century 1769–1865,* volume 1.

Les Presses Universitaires de France: Fauré-Fremiet, E.: *La Cinétique du Développement*; Legendre, R.: *La Concentration en Ions Hydrogène de l'Eau de Mer*; and Terroine, T. F. and Zunz, Edgard: *Le Métabolism de Base.*

Princeton University Press: Conklin, Edwin G.: *Laboratory Directions in General Biology*; McClendon, J. F.: *Physical Chem-*

istry of Vital Phenomena; and Morgan, T. H.: *Evolution and Genetics.*

G. P. Putnam's Son: Beebe, Wm.: *The Arcturus Adventure*; and Beebe, Wm.: *Galapagos—Land's End.*

W. B. Saunders Co.: Dercum, Francis X.: *The Physiology of Mind.*

F. A. Stokes Co.: Noyes, Alfred: *The Book of Earth*; and Noyes, Alfred: *The Watchers of the Sky.*

D. Van Nostrand Co.: Arrhenius, S. A. and Leonard, C. S.: *Chemistry in Modern Life*; Clark, Austin H.: *Animals of Land and Sea*; Greaves, Joseph E. and Greaves, E. O.: *Bacteria in Relation to Soil Fertility*; Luckiesh, M.: *Foundations of the Universe*; Mills, John: *Within the Atom*; Ross, F. E.: *Physics of the Developed Photographic Image*; Tammann and Mehl: *States of Aggregation*; and Whitney, Milton: *Soil and Civilization.*

William Wood and Co.: Fantham, H. B. and Stephens, J. W. W. and Theobald, F. V.: *Animal Parasites of Man*; and Mathews, A. P.: *Physiological Chemistry.*

Yale University Press: Haldane, J. S.: *Respiration*; Jordan, E. O., Whipple, G. C. and Winslow, C. E. A.: *A Pioneer of Public Health*; Morgan, T. H.: *The Theory of the Gene*; and Obermaier, Hugo: *The Fossil Man in Spain.*

The following books were presented by the authors: Allee, W. C.: *Jungle Island*; Keen, William W.: *I Believe in God and in Evolution*; and Agersborg, H. P. K.: *A Laboratory Manual in General Animal Biology.*

The reprints accumulated for the past five or six years, about 13,000 in number, have been catalogued by author and by subject. The cards, amounting to 40,679, have not yet been filed with the cards of the main catalogue, but they will have been before the summer of 1927. The reprints that came from Dr. Whitman's library, approximately estimated as 5,000 unduplicated by our present holdings, were not included, and the cataloguing of these remains to be done in 1927–1928. Current reprints beginning with those issued in 1926, will not be catalogued by subject but will be catalogued by author only. The subject division and the indices of Biological Abstracts will in the future serve the users of the library for this purpose.

The library now contains 18,220 bound volumes which come under the category of books and serials, about seven-eighths being serial volumes. There are, moreover, 38,000 reprints, 13,000 of which were catalogued this year. The number of current serials regularly received is 628; although the actual count shows nearly 1,200 titles in our list of serials, nearly half of these are not now continued or are received at rare intervals. Of the 628 serials coming regularly, 204 are paid by subscription and 424 by exchange with the BIOLOGICAL BULLETIN and by gift.

The budget of the library aside from the special appropriation from the General Education Board totalled the same as in 1925, namely $6,750.00 aside from the regular salaries. The entire amount spent was $6,827.13.

The items under the apportionments as estimated in August, 1925, and as actually spent during the year 1926 are given. The estimated budget was: books, $500; serials, $1,500; binding, $1,000; supplies, $500; service, $50; express, $200; back sets, $3,000. Actually spent, the accounts stand as follows: books, $746.54; serials, $2,730.75; binding, $1,841.44 (current serials, $700.44; back sets, $1,141.00); supplies, $692.23; service, $1,486.22; express, $186.74; and back sets, $352.83.

The increase of the expense of current serial subscriptions is the chief concern of the librarian. Before we secured the special gift from the General Education Board, at the request of the Director of the Laboratory, the Librarian mapped a chart showing a schema for an even and uniform expansion of the library, and the expense attached thereto under each item, through the years 1926–1930. By this scheme the item of "serials" was to change from the requested appropriation for the year 1926, the amount of $1,500, to $2,283; in 1927, to $2,499; in 1928, to $2,742; in 1929, to $2,985; in 1930 and on, to $3,228. It will be noticed that this year (1926) an amount ($2,454.42) was reached which was much more nearly that planned for 1928. It can be seen by referring to last year's report that the same occurred in 1925, for current serials. There are two reasons for this: (1) the increase in prices, and (2) the increase in numbers of the serials we are pressed to secure in current issues. One of two things must be done. A finer discrimination in choice must be exercised to cut out new subscriptions, or more money must be added to this section of the budget.

The librarian begs, therefore, to call for more help from the members of the Corporation and from the Library Committee in selecting the current serials, making a finer discrimination than she can make or else in asking for more money from the Executive Committee for the item of " current serials."

Since we realize that we cannot hold all of the literature in our library that is occasionally called for by our investigators, but that these holdings must be distributed over many libraries, we arranged this summer a meeting of librarians, who, like ourselves, are organizing methods of holding the scientific literature of the world available for the use of scientists. Eleven librarians attended this meeting and the day was given to a discussion of this subject. In the Fall, the librarian attended a meeting of the Eastern College Librarians where the same subject was presented. At this meeting, Dr. Vernon Kellogg and Prof. C. E. McClung presented the subject from the viewpoint of the National Research Council and from the Board of Biological Abstracts respectively.

Word was received on the date of February 11, 1927, that one of the resolutions passed at the meeting in the summer has been arranged, namely, that the Union List of Serials should be continued in current holding for libraries of Canada and of the United States, by the library of Congress.

THE REPORT OF THE DIRECTORS.[1]

To the Trustees of the Marine Biological Laboratory.

Gentlemen: We beg to submit herewith a report of the thirty-ninth session of the Marine Biological Laboratory for the year 1926.

1. *Attendance.*—Reference is made to the lists of Students and Investigators (pp. 27–36) and to the tabular view of Attendance (p. 37). The number of students was held to the usual quotas

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[1] Dr. Lillie resigned as Director August 10, 1926, and Dr. Merkel H. Jacobs was elected in his stead. The Directors' report for 1926 is therefore a joint report.

which led to rather severe competition for admission. A consequence of this was a very high percentage of graduate students in the classes including some who already had the Ph.D. degree. However, as a definite matter of policy, a certain number of places were held open for undergraduate students in order to encourage those of high attainments with definite professional interests. The number of investigators, including research assistants, was 250. The highest previous attendance was 207 in 1925. At the height of the season every working place both in the old and the new buildings was occupied. That this should happen in the second year of use of the new laboratory gives pause for thought. There has been no artificial stimulation of attendance. The demand for places as a result of better equipment and better library will soon be greater than the supply. Under these conditions it is hoped that the new housing conditions referred to below may serve to distribute the attendance over a longer season and so relieve the pressure somewhat during July and the first half of August. A considerable number of investigators work only during a six weeks' period. If these could be distributed so that some could succeed others in the same working places the accommodations of the Laboratory could be made to serve a larger number of persons.

There was a very gratifying increase in attendance of foreign investigators, who came from England (4), Germany (5), Switzerlånd (2), Brazil (1), India (1), Rumania (1), Italy (1), Russia (2), Japan (2), China (1), Philippines (1), Syria (1), representing as many separate universities or research institutions. These investigators bring with them a genuine stimulating influence; and there results a better understanding of the scientific work of the various countries.

2. *Housing Accommodations.*—In the report of the Director for 1925 attention was called to the lack of adequate and suitable housing accommodations under control of the Laboratory for investigators and students. This had become a condition seriously threatening the growth and usefulness of the Laboratory. For about two months in the height of the summer season there was often not a single room available in the village. The uncertainty of obtaining accommodations combined with the unsuitability or high price of any remaining discouraged attendance. The fact

also that the laboratory dormitories were not equipped for other seasons than the summer operated to prevent any who might wish to use the facilities of the laboratories and library at other seasons from doing so. Family accommodations with reasonable sanitary conveniences were conspicuously lacking. In short, the future development of the Laboratory was definitely circumscribed by these conditions.

This matter was accordingly presented to the General Education Board in the autumn of 1925 with a statement of estimated needs; their interest was immediately aroused. The architects of the Laboratory, Messrs. Coolidge, Shepley, Bulfinch, and Abbott prepared attractive preliminary plans as a basis for discussion, and these were presented to the General Education Board in April, 1926. The plans provided for two buildings, one intended primarily for family accommodations with apartments, the other for individual accommodations of dormitory type. Both buildings were planned of fire-proof construction, with complete steam heating equipment in the apartment house and provisions for future installation of such equipment in the dormitory. On May 27th, 1926, after careful consideration, the Members and Trustees of the General Education Board authorized its executive officers in their discretion to commit the Board to an appropriation to the Marine Biological Laboratory of a sum not to exceed $250,000 for the construction and equipment of these buildings.

Final detailed plans and specifications were then prepared, bids secured from several contractors in each trade concerned, contracts let and construction begun early in September.

It is expected that these buildings will be ready for use in June, 1927. Both buildings are in the block next to the laboratories, the apartment house opposite the old lecture hall and the dormitory on the corner diagonally opposite facing the Eel Pond. The dormitory is estimated to accommodate 102 persons, the apartment house about 56, and other buildings used for housing about 132, including employees, a total of about 290 persons. Each of the new buildings, includes a commodious commons room, with fire place, for social purposes. Families of Laboratory workers own 58 houses in Woods Hole, of which 19 are on the Gansett tract. There remain but few lots in the Gansett tract, but the large Devil's Lane tract

MARINE BIOLOGICAL LIBRARY
WOODS HOLE, MASS.

of 105 acres provides amply for many years to come. The village also offers accommodations for a considerable number in the form of houses or rooms for rent.

To Mr. Charles A. Coolidge the Laboratory is indebted for reduction of the usual commission of his firm amounting in value to a gift of over $2,000, thus repeating his generosity in the case of the new laboratory. To him as former trustee, as architect and as benefactor of the Marine Biological Laboratory, we owe a deep debt of gratitude.

The following letter to the General Education Board from a Committee of the Board of Trustees has expressed to them our appreciation of their gifts for the upbuilding of the Marine Biological Laboratory.

August 16, 1926.

President Wickliffe Rose,
General Education Board,
61 Broadway,
New York City.

Dear Dr. Rose: On behalf of the Marine Biological Laboratory and in accordance with the express instructions of its Board of Trustees given at their Annual Meeting on August 10th, 1926, we wish to express our appreciation and thanks for the recent generous gifts made by the General Education Board for the Library and dormitories at Woods Hole.

In an Institution like the Marine Biological Laboratory the Library is a central point of organization, and whatever is done to increase its usefulness is a benefit to every student and investigator. The Library has heretofore lacked many books and serials essential to the investigator, and this has become increasingly felt as the scope of biological research has widened into the neighboring fields of physics and chemistry. With the new resources provided by the gift of the General Education Board, the Library bids fair to become one of the most complete collections of its kind in the world. It will not only supply the needs of those working at Woods Hole, but will also supplement the resources of other institutions. Moreover, it now comes into better position to cope with what might be called the alarming rate of growth of biological literature.

The situation as to living conditions has always been a difficult one at Woods Hole; with the recent extensions of the Laboratory it has become an almost impossible one. Many have come here at a great sacrifice of comfort and convenience for the sake of the opportunity to carry on their studies. The provision of adequate accommodations for students and families will make it possible for many to come who might otherwise be deterred; and will enable those who do come to live free from unnecessary care. Comfortable living quarters will now be available at all seasons, so that the period during which the Laboratory may be used will be greatly extended.

We feel that the new developments made possible by these gifts, following the completion of the new laboratory building, mark an era in the history of the Institution, enabling us to look forward to a period of greatly increased activity, which we hope may serve to justify in some measure the confidence of the Members and Trustees of the General Education Board. We shall be glad to have you transmit our message to your Board, and beg to remain

Yours very sincerely,

Signed by R. G. Harrison,
H. S. Jennings,
F. R. Lillie.

3. *The Report of the Treasurer* shows assets of $2,281,219.79 at the close of 1926; at the end of 1925 the assets of the Laboratory were $2,182,630.50. An income balance of $7.796.31 was carried to current surplus which at the end of the year amounted to $83.503.64. The attention of the members of the Corporation is called to the report for 1925 in which the necessity of maintain-a surplus on current accounts for improvements of a permanent nature is emphasized. The treasurer's report for 1926 shows very clearly that while the income of the Laboratory suffices for current operations it leaves very little for constructive development. The budget of the Laboratory has to be prepared with the very greatest care to avoid a deficit instead of a surplus. It is first outlined for each year in the preceding summer and carefully revised by the Executive Committee. It receives a final revision before the end of the year. Thereafter it is necessary to adhere to it strictly. If income should be shown during the year to be exceeding estimates, leeway is provided for items outside of the budget; otherwise not. A very special responsibility for financial soundness rests upon an organization of scientific men, that their judgment in practical af-

fairs should not prove inferior to their capacity in scientific matters.

4. *The Report of the Librarian* shows what gratifying progress has been made in our aim of building up a strong research library with the aid of our new endowments and the special gifts from the General Education Board acknowledged in the last annual report. Over 3,000 bound volumes and 13,000 reprints were added to those already on the shelves last year, and current serials regularly received were increased from 500 to 628. Back sets have been completed in many cases and gaps filled in. Many gifts of books have been received from publishers and others. The library is in fact just now the most rapidly developing department of the institution, a condition that should be maintained for several years to come. The Library Committee is convinced that more rapid expansion than that originally contemplated can wisely be undertaken in 1927; the General Education Board has accordingly kindly consented to advance $15,000 in 1927 for this purpose instead of $10,000 as originally contemplated out of their gift of $50,000 for library expansion referred to in the report for 1925 (p. 31).

The Ida H. Hyde Scholarship.—This scholarship was named for the first time in the Announcement of the Marine Biological Laboratory for 1927. A permanent endowment of $2,000 has been established by Dr. Hyde in the Marine Biological Laboratory to provide for fees, and contribute to other expenses, of women students or investigators of the University of Kansas working at the Marine Biological Laboratory; it is also provided that if women are not available in any given year, men students or investigators of the University of Kansas become eligible, and if neither women or men from the University of Kansas are available in any year the income may be used to pay the fees of students or investigators from any other educational institution. The older members of the Marine Biological Laboratory do not need to be reminded that Miss Hyde was a regular investigator at the Marine Biological Laboratory in the 'nineties of last century and after that frequently up to the time of the war. Miss Hyde's investigations were then well known. For twenty-two years Miss Hyde was also Professor of Physiology at the University of Kansas.

Her gift thus combines her feeling of loyalty to both institutions, and will serve to perpetuate the memory of her devotion to the progress of science in both places, and to stimulate a like spirit in others.

Changes in Personnel.—At the meeting of the Board of Trustees held on August 10, 1926, the resignation of the Director, Dr. Frank R. Lillie, was accepted and Dr. Merkel H. Jacobs of the University of Pennsylvania, Associate Director of the Marine Biological Laboratory, was elected Director in his stead. Dr. Lillie completed this year thirty-six successive summers at the Marine Biological Laboratory and thirty-four successive years in the service of the Laboratory, as Instructor and Head of the Department of Embryology 1893–1903, as Assistant Director 1900–1907 and as Director 1908–1926. A committee of the Trustees drafted the following letter to Dr. Lillie:

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS.,
August 19, 1926.

PROFESSOR FRANK R. LILLIE,
Woods Hole,
Massachusetts.

Dear Dr. Lillie: With much reluctance, and only at your own particular and repeated request, the Trustees of the Marine Biological Laboratory have accepted your resignation as Director. On behalf of the Board we, the undersigned, appointed by the Trustees to act as their representatives, hereby express our great regret that it has seemed to you necessary to retire from the post which you have so long filled to your own honor and that of the Laboratory.

We shall not here attempt to recite the long record of your contributions to the scientific and material progress of the institution. You have steadfastly upheld the high scientific ideals established during the earlier years of our work. The Trustees appreciate the conspicuous ability, combined with unselfishness, with which you have guided the development of the Laboratory. To you, in large measure, is due the steady broadening in scope and method that has been so conspicuous a feature of its work in recent years. As its Director you began with an institution already rich in achievement but still poverty stricken in respect to material things. You have left it unsurpassed in equipment and endowment, the center of activities that exert an always growing influence on scientific progress throughout the world.

We rejoice that as President of the Corporation your continued participation in the guidance of our enterprise is assured to us. You have known how to achieve efficiency without arousing antagonism, and you have won the sincere respect and friendship of every member of the Laboratory. All the Trustees join in sending you cordial greetings and best wishes for the future, together with the assurance of their warm personal regard.

(Signed) H. S. Jennings,
T. H. Morgan,
Edm. B. Wilson,
Chairman.

To which the following reply was made.

August 25, 1926.

Dr. E. B. Wilson,
Marine Biological Laboratory,
Woods Hole, Massachusetts.

My dear Dr. Wilson: I am very much touched by the message that your committee has conveyed to me on behalf of the Board of Trustees on the occasion of my retirement as Director of the Marine Biological Laboratory. The development of the Laboratory during the period of my Directorship has been due to the union of many fortunate circumstances, chief among which I rank the loyal devotion of Trustees who lent their greater experience and reputation to the efforts of the Director in a spirit of perfect coöperation. Add to this the unsparing generosity of the past President of the Board, the untiring efficiency of the Assistant Director, and the experience combined with loyalty of our technical staff—and a combination is effected that could not be defeated. Patience and faith were the only other necessary factors.

I have no fear for the future so long as the Woods Hole spirit survives, and I am proud and happy in my succession to the Presidency of the Corporation and in the assurance of the continued fellowship of the "old guard."

Faithfully yours,
Frank R. Lillie.

There are included as parts of this report the following addenda:

1. The Staff, 1926.
2. Investigators and Students, 1926.
3. A Tabular View of Attendance, 1922–1926.

4. Subscribing and Coöperative Institutions, 1926.
5. Evening Lectures, 1926.
6. Members of the Corporation, August, 1926.

I. THE STAFF, 1926.

Frank R. Lillie, *Director,* Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

Merkel H. Jacobs, *Associate Director,* Professor of General Physiology, University of Pennsylvania.

ZOÖLOGY.

I. INVESTIGATION

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

E. G. Conklin, Professor of Zoölogy, Princeton University.

Caswell Grave, Professor of Zoölogy, Washington University.

H. S. Jennings, Professor of Zoölogy, Johns Hopkins University.

Frank R. Lillie, Professor of Embryology, The University of Chicago.

C. E. McClung, Professor of Zoölogy, University of Pennsylvania.

S. O. Mast, Professor of Zoölogy, Johns Hopkins University.

T. H. Morgan, Professor of Experimental Zoölogy, Columbia University.

G. H. Parker, Professor of Zoölogy, Harvard University.

E. B. Wilson, Professor of Zoölogy, Columbia University.

II. INSTRUCTION.

J. A. Dawson, Instructor in Zoölogy, Harvard University.

Horace B. Baker, Instructor in Zoölogy, University of Pennsylvania.

E. C. Cole, Assistant Professor of Zoölogy, Williams College.

Rudolf Bennitt, Instructor in Biology, Tufts College.

T. H. Bissonnette, Professor of Biology, Trinity College.

Madeleine P. Grant, Assistant Professor of Zoölogy, Mount Holyoke College.

B. H. Willier, Assistant Professor of Zoölogy, The University of Chicago.

Donnell B. Young, Associate Professor of Biology, University of Arizona.

PROTOZOÖLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

Woolford B. Baker, Associate Professor of Biology, Emory University.

Mary Stuart MacDougall, Professor of Zoölogy, Agnes Scott College.

EMBRYOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

Hubert B. Goodrich, Professor of Biology, Wesleyan University.

Benjamin H. Grave, Professor of Biology, Wabash College.

Charles Packard, Associate in the Institute of Cancer Research, Columbia University.

Harold H. Plough, Professor of Biology, Amherst College.

Charles G. Rogers, Professor of Comparative Physiology, Oberlin College.

PHYSIOLOGY.

I. INVESTIGATION.

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.

Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.

Ralph S. Lillie, Professor of General Physiology, The University of Chicago.

Albert P. Mathews, Professor of Biochemistry, The University of Cincinnati.

II. INSTRUCTION.

Merkel H. Jacobs, Professor of General Physiology, University of Pennsylvania.

William R. Amberson, Assistant Professor of Physiology, University of Pennsylvania.

Wallace O. Fenn, Professor of Physiology, University of Rochester.

Frank P. Knowlton, Professor of Physiology, Syracuse University.

BOTANY.

I. INVESTIGATION.

B. M. Duggar, Professor of Plant Physiology, Washington University.

C. E. Allen, Professor of Botany, University of Wisconsin.

S. C. Brooks, Department of Public Health, Washington, D. C.

Wm. J. Robbins, Professor of Botany, University of Missouri.

J. R. Schramm, Editor-in-Chief, Biological Abstracts, University of Pennsylvania.

II. INSTRUCTION.

Ivey F. Lewis, Professor of Biology, University of Virginia.

Tracy E. Hazen, Assistant Professor of Botany, Barnard College, Columbia University.

William Randolph Taylor, Assistant Professor of Botany, University of Pennsylvania.

LIBRARY.

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.

Assistant Librarian: ——— ———.

Deborah Lawrence, Secretary.

CHEMICAL SUPPLIES.

Oliver S. Strong, Associate Professor of Neurology, Columbia University, *Chemist.*

APPARATUS ROOM.

Samuel E. Pond, Assistant Professor of Physiology, Medical School, University of Pennsylvania, *Custodian of Apparatus.*

SUPPLY DEPARTMENT.

George M. Gray, Curator.

Assistant Curator: ——— ———.

John J. Veeder, Captain.

E. M. Lewis, Engineer.

A. W. Leathers, Head of Shipping Department.

A. M. Hilton, Collector.

J. McInnis, Collector.

A. R. Travis, Collector.

F. M. MacNaught, Business Manager.

Herbert A. Hilton, Superintendent of Buildings and Grounds.

Thomas Larkin, Superintendent of Mechanical Department.

Lester F. Boss, Mechanician.

William Hemenway, Carpenter.

Arnold H. Bisco, Storekeeper and Head Janitor.

2. INVESTIGATORS AND STUDENTS, 1926.

ZOÖLOGY—Independent Investigators.

Allee, W. C., Associate Professor of Zoölogy, University of Chicago.

Armstrong, Philip, Instructor in Anatomy, Cornell University Medical College.

Avery, Bennett F., Adjt. Professor of Anatomy, American Institute of Beirut.

Baker, Woolford B., Associate Professor of Biology, Emory University.

Bennitt, Rudolf, Instructor in Biology, Tufts College.

Bigelow, Robert P., Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.

Bissonnette, Thomas H., Professor of Biology, Trinity College.

Bowen, Robert H., Assistant Professor of Zoölogy, Columbia University.

Breitenbecker, Joseph K., Lecturer in Zoölogy, McGill University.

Bridges, Calvin B., Research Assistant, Carnegie Institution of Washington.

Byerly, T. C., Instructor in Zoölogy, University of Michigan.

Calkins, Gary N., Professor of Protozoölogy, Columbia University.

Chambers, Robert, Professor of Microscopic Anatomy, Cornell University Medical College.

Clark, Eleanor L., Dept. of Anatomy, Medical School, University of Pennsylvania.

Clark, Eliot R., Professor of Anatomy, Medical School, University of Pennsylvania.

Cobb, Nathan A., Technologist and Nematologist, U. S. Department of Agriculture, Washington, D. C.

Cole, Elbert C., Assistant Professor, Dept. of Biology, Williams College.

Cole, Leon J., Professor of Genetics, University of Wisconsin.

Copeland, Manton, Professor of Biology, Bowdoin College.

Covell, Walter P., Assistant, Rockefeller Institute for Medical Research.

Cowdry, Edmund V., Associate Member, Rockefeller Institute for Medical Research.

Cowles, Rheinart P., Associate Professor of Zoölogy, Johns Hopkins University.

Cuajunco, Fidel, Instructor in Anatomy, University of the Philippines.

Cummings, C. E., Director, Buffalo Society of Natural Sciences.

Curtis, W. C., Professor of Zoölogy, University of Missouri.

Dawson, James A., Instructor in Zoölogy, Harvard University.

Dolley, William L., Jr., Professor of Biology, University of Buffalo.

Donaldson, Henry H., Professor of Neurology, The Wistar Institute of Anatomy and Biology.

Folger, Harry T., Instructor, University of Michigan.

Fry, Henry J., Assistant Professor, Washington Square College.

Gabritschevsky, Eugen, Assistant, University of Moscow.

Gates, Frederick L., Associate Member, Rockefeller Institute for Medical Research.

Glaser, Otto C., Professor of Biology, Amherst College.

Goldfarb, A. J., Professor of Biology, College of the City of New York.

Goodrich, Hubert B., Professor of Biology, Wesleyan University.

Gordon, Isabella, 11 Balloch Road, Keith, Banffshire, Scotland.

Grave, Benjamin H., Professor of Zoölogy, Wabash College.

Grave, Caswell, Professor of Zoölogy, Washington University.

Guthrie, Mary J., Assistant Professor of Zoölogy, University of Missouri.

Hance, Robert T., Associate Member, Rockefeller Institute for Medical Research.

Hayden, Margaret A., Assistant Professor of Zoölogy, Wellesley College.

Hisaw, Frederick L., Assistant Professor of Zoölogy, University of Wisconsin.

Hogben, Lancelot I., Assistant Professor of Zoölogy, McGill University.

Hogue, Florence, Assistant Professor of Zoölogy and Physiology, Oregon Agricultural College.

Howland, Ruth B., Assistant Professor of Biology, New York University.

Huettner, Alfred F., Assistant Professor, Dept. of Zoölogy, Columbia University.

Jennings, H. S., Professor of Zoölogy, Johns Hopkins University.

Just, E. E., Professor of Zoölogy, Howard University.

Kindred, James E., Associate Professor of Histology and Embryology, University of Virginia.

Knower, Henry McE., Professor of Anatomy, University of Alabama.

Kunitz, Moses, Associate Member, Rockefeller Institute for Medical Research.

Lancefield, Donald E., Assistant Professor of Zoölogy, Columbia University.

Lancefield, Rebecca C., Assistant in the Department of the Hospital, Rockefeller Institute.

Lehmann, F. E., Osborn Zoölogical Laboratory, Yale University.

Lewis, F. Parke, Buffalo Society of Natural Sciences.

Lillie, Frank R., Professor of Embryology, University of Chicago.

Lynch, Ruth S., Instructor, Johns Hopkins University.

McClung, C. E., Director, Zoölogical Laboratory, University of Pennsylvania.

MacDougall, Mary S., Head, Biology Department, Agnes Scott College.

Marchand, Henri, Chief Preparator, Buffalo Society of Natural Sciences.

Martin, Earl A., Assistant Professor, Department of Biology, College of the City of New York.

Mast, S. O., Professor of Zoölogy, Johns Hopkins University.

Mavor, James W., Professor of Biology, Union College.

Metz, Charles W., Member of Staff, Department of Genetics, Carnegie Institution of Washington.

Morgan, T. H., Professor of Experimental Zoölogy, Columbia University.

Morrill, C. V., Associate Professor of Anatomy, Cornell University Medical College.

Morse, Sterne, Associate in Biophysics, Cleveland Clinic.

Nachtsheim, Hans, Berlin, Germany.

Nassonov, Dimitry, Assistant at the University of Leningrad, Russia.

Nonidez, Jose F., Associate in Anatomy, Cornell University Medical College.

Packard, Charles, Associate, Columbia University, Institute of Cancer Research.

Palmer, G. D., Assistant Professor of Organic Chemistry, Kansas State College.

Parmenter, Charles L., Assistant Professor, University of Pennsylvania.

Pasquini, Pasquale, Assistant at the Zoölogical Institute of the Royal University, Rome, Italy.

Pantin, C. F. A., The Marine Biological Association of the United Kingdom, Plymouth, England.

Plough, Harold H., Professor of Biology, Amherst College.
Popa, Gregor T., Chief-Assistant, University of Bukarest, Roumania.
Rand, Herbert W., Associate Professor of Zoölogy, Harvard University.
Redfield, Helen, National Research Fellow in Zoölogy, Columbia University.
Richardson, Charles H., Entomologist, Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C.
Schrader, Franz, Associate Professor of Biology, Bryn Mawr College.
Schrader, Sally H., Instructor in Biology, Bryn Mawr College.
Scott, Walter J., Graduate Student, University of Pennsylvania.
Scheuring, Ludwig, Professor of Zoölogy and Comparative Anatomy, University of Munich, Germany.
Smith, Fanny F., Research Assistant, Washington University.
Speidel, Carl C., Associate Professor of Anatomy, University of Virginia.
Stern, Curt, Kaiser Wilhelm Institute, Berlin, Germany.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.
Strong, Oliver S., Associate Professor of Neurology, Columbia University.
Tennent, David H., Professor of Biology, Bryn Mawr College.
Uhlenhuth, Edward, Associate Professor of Anatomy, University of Maryland, Medical School.
Weese, Asa O., Professor of Zoölogy, University of Oklahoma.
Whiting, Anna R., University of Maine.
Whiting, Phineas W., Head Department of Biology, University of Maine.
Wieman, Harry L., Professor of Zoölogy, University of Cincinnati.
Wildman, Edward E., University of Pennsylvania.
Williams, Roy G., University of Pennsylvania.
Willier, Benjamin H., Assistant Professor of Zoölogy, The University of Chicago.
Wilson, Edmund B., Da Costa Professor of Zoölogy, Columbia University.
Wilson, James W., Assistant Professor of Biology, Brown University.
Witschi, Emil, University of Basel, Switzerland.
Woodruff, Lorande L., Professor of Protozoölogy, Yale University.
Young, Donnell B., Head Biology Department, University of Arizona.

ZOOLOGY—Beginning Investigators.

Anderson, Pearl, Instructor in Zoölogy, University of Maryland.
Arzberger, E. G., Plant Pathologist, Department of Agriculture, Washington, D. C.
Avery, Margaret S., 1 Marshall St., Ann Arbor, Michigan.
Baker, Herbert N., Johns Hopkins University.
Barth, L. G., Graduate Assistantship, University of Michigan.
Benson, Gertrude C., University of Michigan.
Bergner, A. Dorothy, Columbia University.
Bowen, Edith S., Instructor in Zoölogy and Physiology, Hood College.
Bowling, R., Research Assistant, Columbia University.
Chen, T. Y., Graduate Student, Columbia University.
Christie, Jesse R., Associate Nematologist, U. S. Dept. of Agriculture, Washington, D. C.
Clark, Leonard B., Graduate Student, Johns Hopkins University.

Darby, Hugh H., Instructor, Washington Square College.
Esaki, Shiro, Fellow of the Rockefeller Foundation, Department of Zoölogy. University of Chicago.
Fales, Doris E., Graduate Assistant, Western Reserve University.
Fogg, Lloyd C., Graduate Student, Columbia University.
Gardiner, Mary S., Bryn Mawr College.
Gates, Gordon E., Head Biology Department, Judson College, Rangoon, Burma.
Grant, Madeleine P., Assistant Professor, Mount Holyoke College.
Harnly, Marie L., Carnegie Institution of Washington.
Harnly, Morris H., Graduate Student, Columbia University.
Haynes, Rachel, 32 Fairfield St., Springfield, Mass.
Hickman, Jane F., University of Missouri.
Huggins, J. R., Assistant Instructor, University of Pennsylvania.
Hughes, Thomas P., Assistant, Rockefeller Institute for Medical Research.
Hulpieu, H. R., Graduate Student, Johns Hopkins University.
Johnson, Henry H., Instructor, College of the City of New York.
Karns, Hilda E., Laboratory Assistant, University of Maryland, Medical School.
Koehring, Vera, Instructor in Zoölogy, Smith College.
Long, Margaret E., University of Pennsylvania.
Lucas, Alfred M., Instructor, Washington University.
Lyons, Champ, University of Alabama.
McMullen, Eleanor C., Instructor in Zoölogy, Cornell University.
Manwell, Reginald D., Senior Graduate Assistant, Amherst College.
Mitchell, William H., Jr., Graduate School, Harvard University.
Moses, Mildred S., Research Assistant, Carnegie Institution of Washington.
Oliphant, Dolores, Student, Mount Holyoke College.
Orbison, Agnes M., Assistant Professor of Biology, Elmira College.
Perkins, Earle B., Graduate Student, Harvard University.
Petersen, Walburga A., University of Chicago.
Pollister, Arthur W., Assistant, Columbia University.
Potter, George E., Instructor in Zoölogy, Iowa State University.
Ratcliffe, Francis N., Graduate Student, Princeton University.
Schultz, Jack, Assistant in Zoölogy, Columbia University.
Schwartzbach, Saul, Medical Student, University of Maryland.
Shearer, Edwin M., Graduate Student, Princeton University.
Smith, D. C., Harvard University.
Sonneborn, Morton T., Student, Johns Hopkins University.
Taylor, Ivon R., Instructor, University of Pennsylvania.
Titlebaum, Albert, Columbia University.
Uhlemeyer, Bertha, Assistant Professor, Washington University.
Vicari, Emilia M., Cornell University Medical College.
Wallace, Edith M., Carnegie Institution of Washington.
Whitaker, Douglas M., Graduate Student, Stanford University.
Wolff, William A., Student, University of Pennsylvania.

PHYSIOLOGY—Independent Investigators.

Alvarez, Walter C., Associate Professor of Medicine, Mayo Clinic.
Amberson, W. R., Assistant Professor of Physiology, University of Pennsylvania.

Bodansky, Oscar, Research Assistant in Chemistry. Columbia University.

Bodine, Joseph H., Assistant Professor of Zoölogy, University of Pennsylvania.

Campos, Franklin A. de M., Assistant in Physiology, St. Paul's Medical School, Brazil.

Cattell, McKeen, Instructor in Physiology, Cornell University Medical College.

Chidester, Floyd E., Professor of Zoölogy, West Virginia University.

Collett, Mary E., Assistant Professor of Physiology, Western Reserve University.

DeGraff, Arthur C., Instructor in Physiology, New York University Medical College.

Edwards, Dayton J., Associate Professor, Cornell University Medical College.

Ellsworth, Read McL., Assistant Resident Physician, Johns Hopkins University.

Farr, Clifford H., Associate Professor, Washington University.

Fenn, Wallace O., Professor of Physiology, Rochester University School of Medicine.

Garrey, Walter E., Professor of Physiology, Vanderbilt University Medical School.

Glusker, David, Student, University of Pennsylvania Medical School.

Harvey, Edmund N., Professor of Physiology, Princeton University.

Heilbrunn, Lewis V., Assistant Professor, University of Michigan.

Hemmeter, J. C., Professor Clin. Med., Medical Department, University of Maryland.

Hinrichs, Marie A., The University of Chicago.

Holt, L. Emmett, Jr., Associate in Pediatrics, Johns Hopkins University.

Hoskins, Margaret M., Assistant Professor of Microscopic Anatomy, New York University College of Dentistry.

Inman, Ondess L., Professor of Biology, Antioch College.

Irwin, Marian, Associate in the Division of General Physiology, Rockefeller Institute for Medical Research.

Jacobs, Merkel H., Professor of General Physiology, University of Pennsylvania.

Kahn, Morton C., Instructor in Hygiene, Cornell University Medical College.

Knowlton, Frank P., Professor of Physiology, College of Medicine, Syracuse University.

Landis, Eugene M., Student, University of Pennsylvania.

Lillie, Ralph S., Professor of General Physiology, University of Chicago.

Loeb, Leo, Professor of Pathology, Washington University Medical School.

Lucke, Baldwin, Assistant Professor of Pathology, University of Pennsylvania.

McCutcheon, Morton, Assistant Professor of Pathology, University of Pennsylvania.

Means, James H., Professor of Clinical Medicine, Harvard University Medical School.

Michaelis, Leonor, Resident Lecturer in Research Medicine, Johns Hopkins University.

Mitchell, Philip H., Associate Professor of Physiology, Brown University.

Page, Irvine H., Presbyterian Hospital, New York City.
Peebles, Florence, Pineville, Pennsylvania.
Perlzweig, W. A., Johns Hopkins Hospital.
Plunkett, Charles R., Instructor, New York University.
Pond, Samuel E., Assistant Professor of Physiology, University of Pennsylvania School of Medicine.
Redfield, Alfred C., Assistant Professor of Physiology, Harvard University Medical School.
Reznikoff, Paul, Instructor, Cornell University Medical College.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Woodward, Alvalyn E., Associate Professor, University of Maine.

PHYSIOLOGY—Beginning Investigators.

Baskervill, Margaret, Adjunct Professor, Medical School, University of Texas.
Bewick, William C. D., University Durham, England.
Blumenthal, Reuben, Graduate Student, University of Pennsylvania.
Brown, Dugald E. S., Instructor, New York University.
Cattell, Ware, Research Fellow in Biology, Memorial Hospital.
Choate, Dorothy, Student, University of Pennsylvania.
Coolidge, Thomas, Student, Harvard University Medical School.
De Forest, David McC., Instructor in Biology, Union College.
Farr, Wanda K., Research Worker, Barnard Hospital.
Genther, Ida T., Assistant Instructor, University of Wisconsin.
Grant, Carroll W., Instructor in Bacteriology, Battle Creek College.
Hartline, H. K., Johns Hopkins University Medical School.
Haywood, Charlotte, Graduate Student, University of Pennsylvania.
Hess, Olga T., Leningrad, Russia.
Humphreys, George H., Student, Harvard University Medical School.
Kapp, Eleanor M., Graduate Student, University of Pennsylvania.
Kredel, Frederick E., Student, Johns Hopkins University Medical School.
Kuhn, Harry A., Edgewood Arsenal, Maryland.
Loucks, Milo M., Teaching Fellow in Physiology, University of Minnesota.
Morrison, Mary Elinor, Graduate Student, University of Pennsylvania.
Nadler, Jacob E., Instructor, University of Georgia Medical School.
Nomura, Shichiroku, Assistant Professor of Zoölogy, Biological Institute, Tohoku Imp. University, Sendai, Japan.
Pollock, Herbert, Cornell University Medical School.
Smith, Wilbur A., Research Assistant, University of Pennsylvania.
Sumwalt, Margaret, Graduate Student, University of Pennsylvania.
Thomas, Francisca K., Secretary to Chief of Medical Services, Massachusetts General Hospital.
Thomas, Giles W., Medical Student, Harvard University Medical School.
Wilbur, Lois, University of Pennsylvania.

BOTANY—Independent Investigators.

Brieger, Frederick G., Bussey Institution, Forest Hills, Mass.
Brooks, Matilda M., Associate Biologist, Hygienic Laboratory, Washington, D. C.

Brooks, Sumner C., Biologist, Hygienic Laboratory, Washington, D. C.
Duggar, Benjamin M., Missouri Botanical Garden, St. Louis, Mo.
Hazen, Tracy E., Assistant Professor of Botany, Columbia University.
Keefe, Rev. Anselm M., Professor of Biology, St. Norbert's College.
Lewis, Ivey F., Miller Professor of Biology, University of Virginia.
Taylor, William R., Assistant Professor, University of Pennsylvania.

BOTANY—Beginning Investigators.

Bowman, Paul W., Instructor in Botany, George Washington University.
Rowlee, Silence, Instructor, Wellesley College.
Svenson, Henry K., Assistant Professor of Biology, Union College.

RESEARCH ASSISTANTS—1926.

Arvo, Impi, Union College.
Craighill, Caroline B., Carnegie Institution of Washington.
Downing, Ralph C., Wabash College.
Grand, Constantine, Cornell University Medical College.
Grant, Jean F., Sweet Briar College.
Johnson, Helen R., Brown University.
McNamara, Helen, Rockefeller Institute for Medical Research.
Matthews, Annette, University of Maine.
Spaulding, Janet, Cornell University Medical College.
Wheeler, Philip H., Wesleyan University.
Wilhelm, John F., Jr., Wabash College.
Zimmerman, Averill A., Western Reserve University.

STUDENTS.

1926.

Botany.

Arnold, Dorothy E., Wellesley College.
Borden, Mabel A., Dalhousie University.
Brown, Helen J., Instructor, St. Mary of Springs College, East Columbus, Ohio.
Buehler, Katherine, Teacher, Albany High School.
Clark, Elizabeth B., Student, Radcliffe College.
Cline, Elsie, Student, Johns Hopkins University.
Connard, Mary H., Vassar College.
Copeland, Joseph J., Student, Earlham College.
Gaffney, Catherine A., Hunter College.
Griffin, Grace, Columbia University.
Klinger, Carol, Student, Wabash College.
Lewis, Sara I., Instructor, Northwestern University.
Main, Rolland J., Rutgers College.
Morris, Helen S., Hunter College.
Peterson, Daggmar H., Research Zoölogist, New Jersey Agricultural Experiment Station.

Smith, George H., Instructor, University of Illinois.
Taussig, Joseph, Harvard College.
Turnbull, Virginia E., Teacher, Dorchester High School for Girls.
Vordemberge, Anna M., Goucher College.
Worth, Evelyn W., Teacher, Abington Township, Pennsylvania.

Embryology.

Benner, Miriam C., Mt. Holyoke College.
Brannon, Lida C., Teacher, Dana Hall, Wellesley, Mass.
Britten, Sidney A., Student, Hamilton College.
Campos, F. A. de Moura, Assistant in Physiology, St. Paul's Medical School, Brazil.
Carpenter, Esther, Assistant in Zoölogy, University of Wisconsin.
Chen, Hsin-Kuo, Graduate Student, University of Illinois.
Climenko, David R., Student, Dartmouth College.
Conklin, Cecile L., Teacher, Goucher College.
Cuajunco, Fidel, Instructor in Anatomy, University of the Philippines.
Eggerdink, Anna G., Hunter College.
Else, Frank L., Instructor, University of Pennsylvania.
Esaki, Shiro, Fellow of the Rockefeller Foundation, University of Chicago.
Getchell, Donnie C., Assistant in Biology, Colby College.
Hansen, Ira B., Student, Wesleyan University.
Harrington, John T., Student, Dalhousie University.
Herman, Myra, 1119 Boston Rd., Bronx, N. Y.
Hinchey, Mary C., Instructor, Hollins College.
Holcomb, Mrs. Daisy Y., Instructor, University of Arkansas.
Holmes, Gladys E., Brown University.
Horsley, Guy W., University of Virginia.
Kellicott, Janet, Barnard College.
Lu, Hwei-ling, Huping Christian College, China.
Nelsen, Olin E., Instructor, Toledo University.
Parpart, Mrs. A. K., Graduate Student, Smith College.
Popa, Dr. Gregor T., Chief-Assistant, University of Bukarest, Roumania.
Prew, Paul W., Student, Cornell University Medical College.
Walker, Harriet P., Student, Smith College.
Wu, Chao-Fa, University of Wisconsin.

Attending Morning Lectures Only.

Van Duyne, S. Elizabeth, Physician and Teacher, Goucher College.

Physiology.

Bing, Franklin C., 304 N. Chester Road, Glenolden, Pennsylvania.
Boyd, Marjorie, Instructor, Mount Holyoke College.
De Forest, David McC., Instructor, Union College.
Dowling, Alexander S., Graduate Student, Harvard University.
Dyer, Helen A., Assistant Pharmacologist, Hygienic Laboratory, Wash., D. C.
Field, Madeleine E., Assistant in Physiology, Mount Holyoke College.
Goebel, Walther F., Assistant in Chemistry, Rockefeller Institute, New York City.

Hess, Walter N., Professor of Zoölogy, De Pauw University.
Hitchcock, David I., Associate, Rockefeller Institute, New York City.
Hollander, Franklin, Medical Fellow, National Research Council.
Holmes, M. Thelma, Instructor, Syracuse University.
Kinney, Elizabeth T., Graduate Assistant, Washington University.
MacKay, Margaret E., Assistant in Histology and Embryology, Dalhousie University.
Miller, Helen M., Washington University.
Morton, Harry S., Student, Dalhousie University.
Nomura, Shichiroku, Assistant Professor, Tohoku Imperial University, Sendai, Japan.
Pinkston, James O., Instructor, Birmingham-Southern College.
Robertson, Donald F., University of Missouri.

Protozoology.

Canavan, Wm. P., Instructor, University of Pennsylvania.
Chen, Tse-Yin, Graduate Student, Columbia University.
Crosman, Arthur M., Graduate Student, Columbia University.
Diller, William F., Instructor, Franklin and Marshall College.
Garner, Murvel R., Assistant Professor, Earlham College.
Gelback, Elizabeth L., Assistant in Biology, Goucher College.
Holton, Ruth G., Newburyport, Mass.
Liu, Chung Lo, Foochow, China.
Marsland, Douglas A., Instructor, Washington Square College.
McKinney, Mary Ann, Assistant Professor, Stephen F. Austin State Teachers College.
Parpart, Arthur K., Graduate Assistant, Amherst College.
Seton, Henry, Columbia University.
Severinghaus, Aura E., Assistant in Zoölogy, Columbia University.
Showers, Edith, Smith College.
St. John, Joe H., Instructor, Army Medical School, Washington, D. C.
TeWinkel, Lois E., Assistant in Zoölogy, Barnard College.
West, Luther S., Professor of Biology, Battle Creek College.
Willey, Charles H., Instructor, New York University.
Zeek, Pearl M., Medical Student, Columbia University.

Zoology.

Avery, Bennett F., Adjt. Professor of Anatomy, American University of Beirut.
Beale, Alice, Radcliffe College.
Bean, Ralph C., Teacher, Girls' High School, Boston, Mass.
Beaver, Paul C., Student, Wabash College.
Chase, Aurin M., Amherst College.
Clarke, George L., Harvard University.
Crawford, Wiley W., Graduate Assistant in Zoölogy, University of Missouri.
Daniel, George E., Student, University of Arkansas.
Dawley, Charlotte, Graduate Assistant, Washington University.
Drayer, Calvin S., Student, Ohio Wesleyan University.
Duryee, William R., Yale University.

East, Elizabeth W., Wellesley College.
Farr, Marion M., Zoölogy Laboratory Assistant, Vassar College.
Hahnert, William F., Student, De Pauw University.
Hardesty, Mary, Sophie Newcomb College.
Herskowitz, Isidor A., Student, Columbia University.
Hewes, Edna M., University of Rochester.
Hummel, Katherine P., 2143 Commonwealth Avenue, St. Paul, Minnesota.
Hunt, Thomas E., Jr., University of Chicago.
Hurlbutt, Ellen L., Instructor, Connecticut College.
Irwin, Marion S., Instructor in Zoölogy, Carleton College.
Jeffers, Katharine R., University of Missouri.
Maclean, Bernice L., Assistant, Zoölogy Department, Mt. Holyoke College.
Markle, Millard S., Professor of Biology, Earlham College.
McCoy, Oliver R., Graduate Assistant, Washington University.
McGaun, Ralph C., Jr., Student, Amherst College.
McMullen, Donald B., Graduate Assistant, Washington University.
Miller, Raffael McV., Student, Lafayette College.
Nahm, Laura J., Assistant in Zoölogy, University of Missouri.
Parker, Raymond C., Assistant in Biology, Yale University.
Parsons, Elizabeth H., Student, Oberlin College.
Payne, Nellie M. de C., University of Pennsylvania.
Perrine, Ruth R., Oberlin College.
Pierce, Madelene E., Radcliffe College.
Pike, Melvin H., University of Michigan.
Reinhard, Edward G., Buffalo Society of Natural Sciences.
Rich, Robins, Sweet Briar College.
Robb, Robert C., Assistant, Dalhousie University.
Rogers, Edith, Goucher College.
Rossmeisl, Elsie C., Smith College.
Rowell, Lyman S., Instructor, University of Vermont.
Schwind, Joseph L., Assistant in Biology, Yale University.
Sears, Mary, Radcliffe College.
Shattuck, George E., Wesleyan University.
Shields, M. Lawrence, Instructor in Biology, Phillips Academy.
Sloan, Eleanore, Student, Wilson College.
Thompson, Margaret H., Knox College.
Underwood, Katharine, Woods Hole, Mass.
Van Cleave, Charles C., Assistant, University of Chicago.
Walls, Gordon L., Tufts College.
Warbasse, Agnes, Barnard College.
Weston, Dorcas, 3 Center St., Portland, Maine.
Wolf, Opal M., Assistant in Zoölogy Department, University of Wisconsin.
Woods, Farris H., Instructor, University of Missouri.
Wormley, Lowell C., Dartmouth College.
Wright, Stillman, Fellow in Zoölogy, University of Wisconsin.

3. TABULAR VIEW OF ATTENDANCE.

	1922	1923	1924	1925	1926
INVESTIGATORS—Total	182	176	194	207	240
Independent:					
Zoölogy	87	90	77	84	107
Physiology	28	23	33	40	41
Botany	15	13	14	11	8
Under Instruction:					
Zoölogy	34	41	47	45	53
Physiology	11	5	16	23	28
Botany	7	4	7	4	3
STUDENTS—Total	126	146	134	132	141
Zoölogy	59	59	50	54	56
Protozoölogy	12	16	17	17	19
Embryology	28	31	29	29	28
Physiology	19	22	18	19	18
Botany	8	18	20	13	20
RESEARCH ASSISTANTS					12
TOTAL ATTENDANCE	308	322	328	339	393
Less eight registered as both Students and Investigators					8
					385
INSTITUTIONS REPRESENTED—Total	104	107	110	112	119
By investigators	71	62	69	74	84
By students	68	73	68	65	60
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	—	—	—	1	—
By students	—	4	—	4	4
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	—	—	—	—	17
By students	—	—	—	—	3

4. SUBSCRIBING AND COOPERATING INSTITUTIONS, 1926.

American Association for Advancement of Science
Amherst College
Antioch College
Battle Creek College
Bowdoin College
Brown University
Bryn Mawr College
Buffalo Society of Natural Sciences

Carnegie Institution, Cold Spring Harbor
Carnegie Institution of Washington
Chinese Educational Mission
Columbia University
Commonwealth Fund
Cornell University
Cornell University Medical College
Dalhousie University
Dartmouth College
De Pauw University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
International Education Board
Johns Hopkins University
Johns Hopkins University Medical School
Knox College
Lafayette College
Eli Lilly & Co.
Massachusetts Institute of Technology
Mount Holyoke College
National Research Council
New York University
New York University, Dental School
New York University, Medical School
Oberlin College
Ohio Wesleyan University
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Smith College
Sophie Newcomb College
Tufts College
Union College
United States Army, Med. Dept.
United States Dept. of Agriculture
University of Chicago
University of Cincinnati
University of Georgia, Medical School
University of Illinois
University of Iowa
University of Maryland
University of Maryland, Medical School
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania, Medical School
University of Rochester
University of Vermont
University of Virginia
University of Wisconsin
Vassar College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Western Reserve University
Wistar Institute of Anatomy and Biology
Yale University

SCHOLARSHIP TABLES.

Lucretia Crocker Scholarships for Teachers in Boston.
Scholarship of $100 supported by a friend of the Laboratory since 1898.
Ida H. Hyde Scholarship of the University of Kansas.
The Edwin S. Linton Memorial Scholarship of Washington and Jefferson College.

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5. EVENING LECTURES, 1926.

Tuesday, June 29,
Professor Raymond Pearl . . . . . "Alcohol and Longevity."

Friday, July 2,
Dr. G. E. Coghill . . . . "The Early Development of Behavior and Related Nervous Structures in Amblystoma."

Tuesday, July 6,
Professor Alfred C. Redfield . . "The Respiratory Proteins of the Blood."

Friday, July 9,
Dr. Robert Chambers, assisted by Dr. Paul Reznikoff . . . . . . "Some Aspects of the Reaction of Protoplasm to Salts." With Demonstration of the Micrurgical Technic on Living Cells Projected on the Screen.

Tuesday, July 13,
Dr. C. W. Metz . . . . "Aberrant Features of Chromosome Behavior and Genetic Behavior in Sciara and the Problems They Present."

Friday, July 16,
Dr. H. S. Jennings . . . . "Biology and Experimentation."

Tuesday, July 20,
Dr. W. O. Fenn . . . . "Some Problems Concerned with the Shortening of Muscles."

Thursday, July 22,
Professor William Patten . . . . . "Hunting for Missing Links in Spitzbergen."—Illustrated.

Friday, July 23,
Dr. E. Witschi . . . . "Heredity and Environment in the Sex-determination."

Tuesday, July 27,
The William Thompson Sedgwick Memorial Lecture, delivered by Professor T. H. Morgan .... "Genetics and the Physiology of Development."

Friday, July 30,
Professor L. Michaelis........ "The Properties of Certain Artificial Membranes as a Model for Cell Membranes."

Tuesday, August 3,
Dr. Marshall A. Howe........ "Reef-building and Land-forming Plants."—Illustrated.

Friday, August 6,
Dr. H. S. Reed................. "The Absorption of Kations and Anions by Plant Roots."

Friday, August 13,
Dr. H. D. Fish................ "Biology Students in British Guiana."—Illustrated.

Thursday, Sept. 9,
Dr. Hans Driesch............ "The Problem of Freedom."

6. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.

Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Miss E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.
Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J.
Osborn, Prof. Henry F., American Museum of Natural History, New York City, N. Y.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Commercial Museum, Philadelphia, Pa.

2. Regular Members, August, 1926.

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Mass.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.

Adolph, Dr. Edward F., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Agersborg, Dr. H. P. K., James Millikin University, Decatur, Illinois.

Allee, Dr. W. C., University of Chicago, Chicago, Illinois.

Allen, Prof. Chas. E., University of Wisconsin, Madison, Wisconsin.

Allen, Prof. Ezra, Carnegie Institution of Washington, Cold Spring Harbor, Long Island.

Allyn, Dr. Harriet M., Vassar College, Poughkeepsie, N. Y.

Amberson, Dr. William B., University of Pennsylvania, Philadelphia, Pa.

Anderson, Dr. E. G., University of Michigan, Ann Arbor, Michigan.

Atterbury, Mrs. Ruth R., Great Neck, Long Island, New York.

Baitsell, Dr. George A., Yale University, New Haven, Conn.

Baker, Dr. E. H., 5312 Hyde Park Boulevard, Hyde Park Station, Chicago, Ill.

Baldwin, Dr. F. M., Iowa State College, Ames, Iowa.

Bascom, Dr. K. F., Medical School of Virginia, Richmond, Virginia.

Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, N. Y.

Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, La.

Bennitt, Dr. Rudolf, Tufts College, Tufts College, Mass.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Mass.

Binford, Prof. Raymond, Guilford College, Guilford College, N. C.

Bodine, Dr. J. H., University of Pennsylvania, Philadelphia, Pa.

Boring, Dr. Alice M., Yenching College, Peking, China.

Bowen, Dr. Robert H., Columbia University, New York City.

Box, Miss Cora M., University of Cincinnati, Cincinnati, Ohio.

Bradley, Prof. Harold C., University of Wisconsin, Madison, Wis.

Brailey, Miss Miriam E., 800 Broadway, Baltimore, Md.

Bridges, Dr. Calvin B., Columbia University, New York City.

Brooks, Dr. S. C., U. S. Public Health Service, Washington, D. C.

Buckingham, Miss Edith N., 342 Marlboro St., Boston, Mass.
Budington, Prof. R. A., Oberlin College, Oberlin, Ohio.
Bumpus, Prof. H. C., Duxbury, Mass.
Byrnes, Dr. Esther F., 1803 North Camac St., Philadelphia, Pa.
Calkins, Prof. Gary N., Columbia University, New York City.
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BIOLOGICAL BULLETIN

ANATOMY AND FUNCTION OF THE REPRODUCTIVE SYSTEM IN THE SNAIL, *LYMNÆA STAGNALIS APPRESSA* SAY.[1]

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Introduction.

Since the work of Baudelot ('63) is apparently the only available source of information on the finer anatomy of the reproductive system of the European pond snail, *Lymnæa stagnalis* Lin., and since no definite description of the confluence of the hermaphrodite duct with the male and female conduits has been found for the American sub-species, *L. s. appressa* Say, it was deemed advisable to undertake to describe this system in the latter form in the hope that the effort would contribute to the solution of the general problem of reproduction in hermaphroditic fresh-water snails. Elsewhere the writer has given the results of his investigation of this problem by genetical (Crabb, '27*a*) and cytological methods (Crabb, '27*b*). In the former paper it is shown that the distinguishing characters were not inherited, but in the latter paper it is shown that self-fertilization is the normal mode of reproduction in isolated *L. s. appressa* Say.

It is interesting to note that the anatomy of the gastropod reproductive system was not understood until well into the nineteenth century. Baudelot ('63) points out that Muralt, 1677, Redi, 1684, Lister, 1694, Swammerdam, 1737, Cuvier, 1802–09, Owen, 1825, and several others published descriptions of the reproductive system of various hermaphroditic gastropods in which they held that the sex glands are separate organs instead of forming an ovotestis. That is, they failed to recognize the dual nature of the ovotestis. Since these observers believed the gastropods to be unisexual they mistook some accessory organ for an ovary or a testis. For instance, Wagner ('35) points out that Cuvier, Meckel, Carus, Delle and others considered the albumen gland as one part and the prostate gland as another part of the testis in several gastropods, while Prevost and some others regarded the ovotestis as the testis and the albumen gland as the ovary in a species of *Lymnæa*. Cuvier ('46) holds that in *Lymnæa* and *Planorbis* the ovary and testis are separate, the former sometimes being imbedded in the anterior part of the liver while the latter is near the apex of the shell. Baudelot ('63) states that Van Beneden, 1838, mistook the egg membrane gland for the testis in *Lymnæa glutinosus*. Paasch ('43) called the ovotestis a testis, and the hermaphrodite duct the epididymis in *Planorbis corneus, Lymnæa stagnalis, L. palustris, L. auricularium* and *L. elongatus*. Simroth ('87) thought he found "pure" functional females, *i.e.*, individuals lacking the prostatic apparatus, in *Agriolimax*. The relative frequency with which he found these "pure" females justifies the criticism of a contemporary investigator, Schiemenz ('88), who states that Simroth failed to find the male organ because one organ lies within the other. Hofmann ('12) describes pathological and other abnormal conditions in *Helix* in which various parts of the sex organs are wanting, rudimentary, doubled or even trebled, but attaches no significance to them.

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

Wagner ('35, p. 104) appears to be the first worker to suspect the true nature of the ovotestis in *Gastropoda,* while Baudelot ('63) is the first to have given an intelligible description of the finer anatomical relation of the parts of *Lymnæa* in that region of the reproductive system where the hermaphrodite duct empties into the definite male and female conduits and the albumen gland duct empties into the uterus.

This work was done under the direction of Professor A. Franklin Shull, and the snails used were identified by Dr. Bryant Walker and Mr. W. J. Clench.

The two figures of the reproductive system of *L. s. appressa* represent the result of information obtained by dissecting twenty-six individuals, several of which were variously prepared and injected with colored solutions, and of sectioning various parts of the entire system of others. In view of individual variations and for the sake of clarity many of the convolutions were omitted in Fig. 1. This is especially true in the case of the hermaphrodite duct and convoluted uterus (*CU*); however, with these exceptions, and that of the copulatory organ, all structures were outlined with projecting apparatus.

Description.

The reproductive system of *L. s. appressa,* like that of most of the hermaphroditic pond snails, comprises three major divisions: (*a*) the hermaphrodite apparatus which includes the ovotestis and hermaphrodite duct; (*b*) a female part including the oviduct proper, the uterus with its convoluted part, albumen gland, egg membrane gland and egg mass membrane gland, the vagina with which the duct of the sperm receptacle is confluent and the sperm receptacle and (*c*) the male part including the vas efferens, prostate gland, vas deferens and copulatory organ. The male and female parts of the reproductive system are quite distinct morphologically and histologically, yet they are intricately appressed and entwined except for the distal half of the vas deferens and the copulatory organ. Functionally, they are similar inasmuch as it is apparent that both ova and sperms may pass out either the male or the female part.

The hermaphrodite gland or ovotestis is a complexly branched, more nearly digitate than acinous, gland, imbedded in the tissue of the liver, though not entirely surrounded by it. Its long axis conforms to that of the liver and sends irregular lobes toward the convex sides of this organ or quite to its periphery (*HG*). The apical end of the ovotestis lies within the mid-region of the third whorl of the shell (*C*), and part of its surface is normally exposed on the concave side, due to its not being covered entirely by liver tissue in this region. Incidentally, there is no hepatic tissue in the apex of the liver beyond the region marked *C* in Fig. 1, nor in certain other areas, chiefly peripheral. Baudelot ('63) describes the form of the ovotestis in *L. stagnalis* as being nearly like that of an elongated triangle and F. C. Baker ('11) figures it as being fusiform in *L. s. appressa.* This certainly is not the case in my material, for I have dissected the liver tissue away in five specimens and found the ovotestis (*HG*), to be very irregular in form.

Investigators apparently are not in agreement as to whether the form and arrangement of the tubules of the ovotestis in some of the more common gastropods is an acinous or a branched digitate gland. On the one hand, some of the forms reported to have an acinous gland are *Helix arbustorum* (Buresch, '12) ; *Limax maximus* (Hoffman, '22) ; *Limax cinera* and *Lymnæa stagnalis* (Baudelot, '63). On the other hand, Gatenby ('17) states that the ovotestis of *Helix aspersa* is formed of "finger-like diverticula" which connect with the hermaphrodite duct; Baudelot ('63) describes the ovotestis of *H. pomatia* as a branched gland having simple digitate terminal tubules, Ancel ('02) appears to be in accord with him since he uses the term "culs-de-sac" and F. C. Baker ('00) states that in *L. emarginata,* var. *mighelsi* "the ovotestis is made up of a number of rounded or lobulated follicles." In *L. s. appressa,* although the ovotestis is composed of a great number of convoluted tubular parts, which are reproductive practically throughout, and although the lumina frequently are irregularly distended in places, there are no typical terminal acini. For this reason it should be referred to the compound branched type of gland, but for the sake of clarity the custom of calling the tubules "acini" will be followed.

The hermaphrodite duct proper (*HD*) is a very complexly lobulated and convoluted tube which is flexed upon itself in three or more places when the animal is contracted. It originates by the confluence of a number of lateral tubules, which are most noticeable in that part of the ovotestis from *G* to beyond *H,* and continuing cephalad within the tissue of the gland, it becomes a free duct at point *H.* This makes it necessary for those ova and sperms which are formed in the anterior portion of the gland to travel toward its apex in order to reach the hermaphrodite duct proper. The enlarged part of the duct, especially that lying between *H* and *O*, functions as a seminal vesicle, and contains sperms at all times in normal adults. Near its anterior end the hermaphrodite duct becomes simple and filiform; then bifurcating sends a limb (*VE*) directly to the posterior end of the prostate gland and another (*OV*) apparently to the duct of the albumen gland (*AGD*); or in other individuals, directly to the convoluted part of the uterus, tangent, but not confluent with the albumen gland duct. Thus in the former case the anterior part of the duct of the albumen gland serves as a common duct (*CD*) for the passage of both ova and albumen directly into the convoluted part of the uterus at a point near its origin. Even though the two ducts were not confluent and entered the uterus side by side, as some of my material seems to show, and as Baudelot ('63, p. 192, Pl. IV.) holds for *L. stagnalis* and *Planorbis corneus*, it would make little or no difference in the time of contact of the ova with the albumen. What effect this albumen has on the movements of sperms in the uterus has not been demonstrated by experimental means. However, masses of sperms enveloped in the albumen of *L. s. appressa* and *L. palustris* eggs are unable to move with sufficient freedom to extract their tails from those of other sperms. The entanglement of the sperm tails is apparently not due to normal agglutination, but to chance entanglement during or prior to passage through the oviduct. This observation argues against insemination occurring in the presence of any quantity of albumen.

Although Klotz ('89) working on *Lymnæa ovatus*, with which he found the work of H. Eisig, 1869, on *L. auricularia* to be in very close agreement, does not bring out the relationship of these ducts definitely; it appears that in his Fig. 1 the hermaphrodite duct terminates in these two species in a manner quite comparable to that of *L. s. appressa*. Moquin-Tandon ('55), Cook ('95), Meisenheimer ('21) and Paasch ('43) on *L. stagnalis* and F. C. Baker ('11) on *L. s. appressa* do not describe the relation of the parts in this region of the reproductive system. Colton ('12) states that in *L. columella* the albumen gland opens into the hermaphrodite duct before the latter divides to join the male and female conduits. Lacaze-Duthiers ('99) clearly shows that in *Ancylis fluviatilis* the limb of the hermaphrodite duct enters the uterus at a point decidedly anterior to the entrance of the albumen gland duct.

Much discussion has arisen concerning the structure of the anterior part of the hermaphrodite duct which would enable it to separate the ova from the spermatozoa. Moquin-Tandon ('55) in speaking of the hermaphroditic Gastropoda in general, says that the eggs are caused to fall into the uterus by escaping between the lips of the longitudinal groove (presumably in the hermaphrodite duct). He infers that this mechanism prevents sperms from entering the uterus as well as eggs from entering the vas efferens. Lacaze-Duthiers ('99) states that in *Ancylis* it is possible to find sperms in the upper part of the uterus; but if they are found there it is an exception. He advances the theory that while a snail is functioning as a male, its oviduct is closed and thus prevents the entrance of sperms. Likewise while the animal is functioning as a female only the female conduit is open; therefore no special structure is necessary to insure separation of eggs and sperms. Owing to the continual presence of sperms in the hermaphrodite duct and ovotestis, this idea is wholly untenable for *L. s. appressa.* He refers to a statement made by Dubreuil, 1873, that in *Helix* the ova fall into the oviduct while the sperms pass on down the male part. It would seem that any opening through which an ovum could fall would certainly admit sperms which are very much smaller and motile.

I have not been able to find any special device for separating the eggs from the spermatozoa in *L. s. appressa,* but in sectioned tissue I have found ova which had passed through the male conduit during copulation and had been injected into the sperm receptacle along with the sperms, and I have commonly found masses of sperms in the albumen of the eggs of *L. s. appressa* and *L. palustris* which were isolated as embryos and kept in strict isolation. Wierzejsky ('05) found masses of sperms in eggs of *Physa fontinalis.* Of three *L. s. appressa* sperm receptacles fixed at the time of copulation, one contained several ova. Since these ova had no albumen around them, it is clear that they passed from the acting male into the sperm receptacle of the acting female. Things have often been seen in the albumen of sectioned eggs of *L. s. appressa* which probably were pieces of single sperm tails and heads. From the foregoing account it is apparent that spermatozoa are usually passed out with ova and subsequently enveloped in the albumen by the egg membrane, and that ova sometimes pass out through the copulatory organ and are injected into the sperm receptacle along with the spermatozoa.

The albumen gland (*AG*) is closely appressed to nearly the entire length of the convoluted uterus. It supplies the albuminous portion of the egg. Its short duct (*AGD*) empties into the convoluted part of the uterus near the most posterior point in its lumen. Keferstein ('62–'66) believed that the albumen gland is wanting in some water snails.

The convoluted part of the uterus (*CU*) is intricately folded upon itself and has regions of large granular cells and others of ciliated columnar epithelial cells. The regions of large glandular cells are the more extensive and occupy most of the interior part of this organ leaving only a relatively small lumen. This part of the reproductive system forms a distinctly blind pounch in some, perhaps most, forms of the Stylommatophora. In *Helix pomatia* (Meisenheimer, '07) *Limax maximus* (H. Hoffman, '22) and probably in *Limax cinera* and *Arion* (Baudelot, '63) this pouch functions as a "fertilization pocket." This does not appear to be the case in any of the Basommatophora.

The egg membrane gland (*EMG*) nearly envelops the convoluted uterus at the point where it ceases to be externally convoluted and becomes uniformly glandular. This gland secretes the gelatinous material in which the individual eggs are imbedded, as Baudelot has shown.

The mass membrane gland (*MMG*) is supposed by Baudelot to be a reservoir for eggs and gelatin. He thinks that the egg mass is formed within it. My observations also point to its having this function. Cunningham ('99) believes that the mass membrane is supplied by the sole gland. This assumption is based upon observations made on marine forms, *Buccinum* and *Murex*. In a specimen of *L. s. appressa*, eggs which had been extruded through a wound in the duct just before it enters this gland were completely formed, but the mass membrane was entirely wanting. There were also free eggs within this region of the duct and one within the lumen of the mass membrane gland itself. Since this snail was fixed in a chromic-potassium bichromate solution before being dissected, these eggs were entirely free from gelatin, hence contributed no information to the question whether or not any of this material is formed by the mass membrane gland. Since I found a fully formed mass of eggs in the vagina of *L. columella*, it appears that no gelatin is contributed by this gland. These observations indicate that the mass membrane gland probably secretes only the membrane which envelops the entire mass of eggs and thus they support Baudelot's conclusions regarding the functions of this gland.

The vagina (*VG*) is here considered that part of the female system which extends from the mass membrane gland to the female orifice (*FO*). It is lined with ciliated epithelial cells and does not possess any cells of the large glandular type. Its external orifice (*FO*) is on the right side of the animal, at a point about midway between the male orifice (*MO*) and the pulmonary orifice (*PO*) when the snail is normally extended.

The sperm receptacle or copulatory pocket (*SPR*) is a blind, thin-walled pouch, lined with ciliated columnar epithelial cells and capable of great distention. It is provided with a narrow duct (*SPRD*) which is confluent with the vagina at a point (*SPO*) very near the female orifice. It functions during copulation as a receptacle for free-swimming spermatozoa, most of which disintegrate or are ejected within four hours after copulation. Animals killed after having functioned as females for fifteen to thirty minutes have the receptacle enormously distended with fluid and spermatozoa. Although there is nothing to warrant considering this sac an actual gland, as H. B. Baker ('25*a*) suspects is the case in *Lanx,* it is capable of self lubrication and probably secretes the thick yellowish fluid which occurs within it in virgins and individuals that have not mated recently.

Instances of variations in which this receptacle is directly connected with the vas deferens have been reported. Kleinert (Robson, ,'23, Diver, '25) for *Heliz hortensis* and Remanujam ('22) for Vaginulidæ, state that in these forms the sperm receptacle is directly connected with the vas deferens, while Paluszenski ('10) found in *Helix pomatia* a short diverticulum on the duct of the receptaculum which he considers a vestigial connection with the oviduct. H. B. Baker ('25*b,* Fig. 24) shows a distinct connection between the male and female conduits in *Hendersonia.* Hoffmann ('12), Cerńy ('07), Stubs ('98) and others have ascribed such modifications to congenital, regenerative or pathological abnormalities in the species which they studied.

The prostatic or posterior part of the male system is a greatly enlarged, cavernous and highly glandular conduit (*PG*) which receives the filiform vas efferens into its posterior end and gives off a short, thick, glandular tube, the beginning of the vas deferens, from its globular anterior end.

The vas deferens proper is a filiform, muscular tube lined with ciliated columnar cells. Originating as a thick tube at the anterior end of the prostate gland, it describes a tortuous course and eventually enters the copulatory organ (*COI*) where it terminates in a short needle-pointed intromittent organ (*IO*). That part of the vas deferens lying between the copulatory organ and the male orifice is free within a cavity of the body, while that between the male and female orifices lies just under the body wall. In the living snail this tube is visible from its attachment at the female orifice to its insertion in the copulatory organ and during copulation it can be seen very distinctly moving in strong undulations. Excised pieces of the living vas deferens writhe like pieces of a living earth worm, thus demonstrating a function of its well-developed muscular walls.

At the time of copulation the copulatory organ is evaginated through the male orifice, and after becoming turgid presents a conical end with the intromittent organ extended as in Fig. 2.

Conclusions.

The results of this investigation show that the anatomy of the reproductive system in *L. s. appressa* is very similar to that in *L. stagnalis,* as described by Baudelot. However, it is also shown that cross-fertilization probably is not the normal mode of reproduction in either form. Spermatozoa are placed in the sperm receptacle during copulation and in order to inseminate the ova they must swim the length of the female conduit, through the viscous substances secreted by the albumen gland, egg membrane gland and mass membrane gland and penetrate the ovum before it is covered with albumen. This would be not later than the arrival of the ovum at the convoluted uterus. Whether these viscous secretions prevent or even hinder the movements of the spermatozoa is not known. However, sperms in the albumen of *L. palustris* eggs which have been oviposited are apparently helpless with regard to locomotion.

From the foregoing explanations it is apparent that in the process of fertilization foreign sperms have little or no chance to compete successfully with sperms which develop and ripen in the same acinus with the egg. Therefore one could hardly expect cross-fertilization to be the normal method of reproduction in *Lymnæa stagnalis appressa.*

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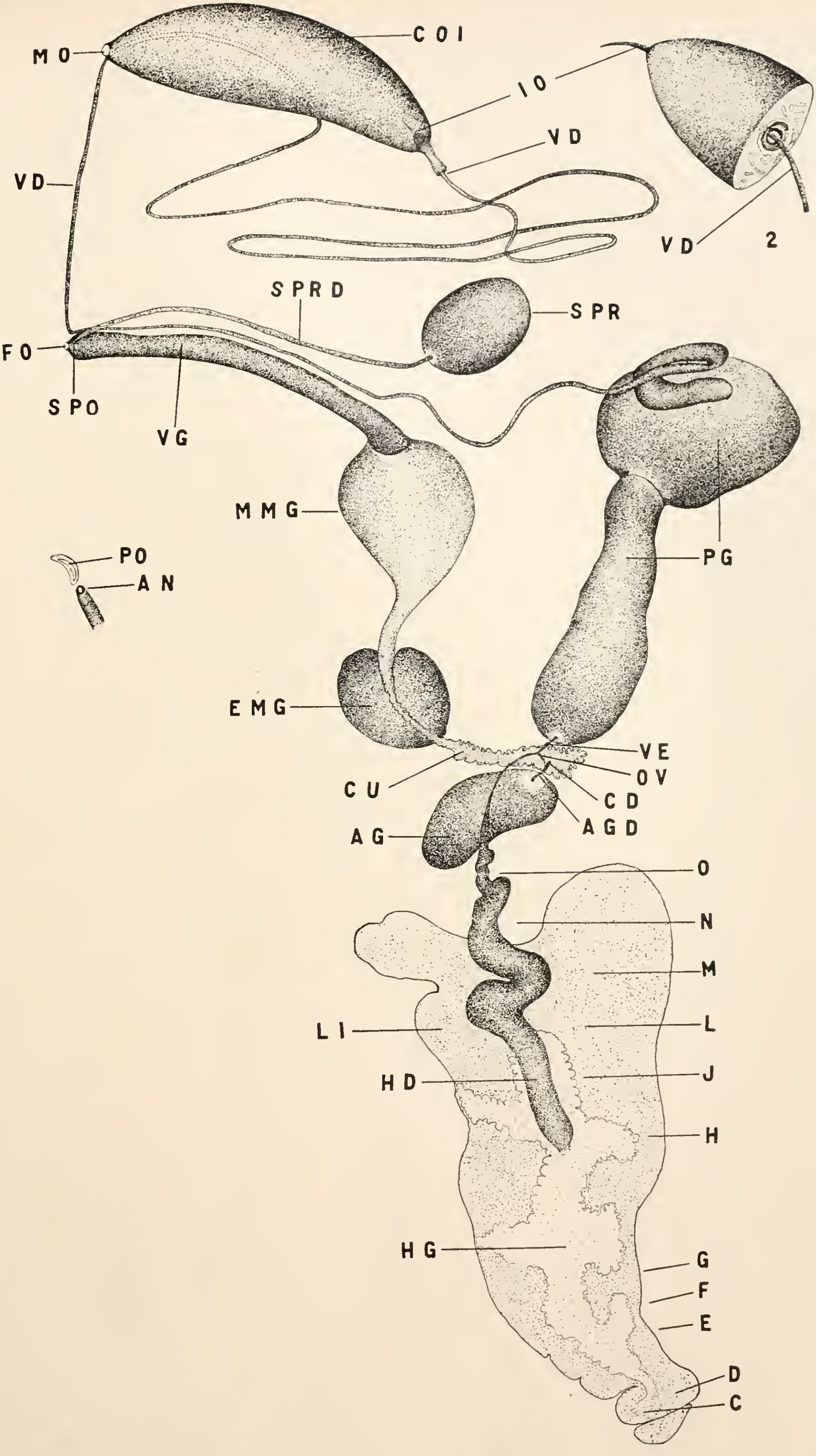
Plate I.

Fig. 1. Reproductive system of *Lymnæa stagnalis appressa* Say, viewed from the ventral side, with parts distended and partly diagrammatized.

AN, anus; *AG,* albumen gland (albuminiparous gland, F. C. Baker, '11); *AGD,* albumen gland duct; *C* to *O,* regions in the hermaphrodite gland and duct in which ovarian or free, ripe ova were found; *CD,* common duct of the albumen gland and of the oviduct; *COI,* copulatory organ invaginated; (larger sac of penis, H. B. Baker, '25); *CU,* convoluted part of uterus (uterine portion of oviduct, F. C. B., '11); *FO,* female orifice; *HD,* hermaphrodite duct (ovisperm duct, H. B. B., '25); *HG,* hermaphrodite gland or ovotestis; *IO,* intromittent part of copulatory organ (verge, H. B. B., '25), [not fully retracted, Fig. 1, extended, Fig. 2]; *LI,* liver; *MMG* mass membrane gland (first accessory albuminiparous gland, F. C. B., '11); *MO,* male orifice; *OV,* oviduct proper; *PG,* prostate; *PO,* pulmonary orifice; *SPO,* sperm receptacle duct-orifice; *SPR,* sperm receptacle (spermatheca, F. C. B., '11; bursa, H. B. B., '25); *SPRD,* sperm receptacle duct; *VD,* vas deferens; *VG,* vagina (free portion of oviduct; vagina is *SPO* to *FO,* my Fig. F. C. B., '11). *MO, FO, PO* and *AN* are represented in their proper spatial relationship as regards each other and the entire reproductive system.

Fig. 2. Excised tip of evaginated copulatory organ, reconstructed from living and sectioned material, freehand drawing, labeling as above.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EDWARD DRAKE CRABB.

THE FERTILIZATION PROCESS IN THE SNAIL, *LYMNÆA STAGNALIS APPRESSA* SAY.[1]

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INTRODUCTION.

The primary object of this study is to determine whether hermaphroditic pond snails which have been reared and kept in strict isolation reproduce by self-fertilization or by parthenogenesis. On this point previous investigators have reached different conclusions. The earliest observations on the manner of reproduction in hermaphroditic gastropods available are those of Aristotle who states that the individuals of the group of Testacea (which included Gastropoda) reproduce like plants, the inference being that they spring from the mud or water. Strange as it may seem, according to Baudelot ('63) no one appears to have questioned Aristotle's views until about the end of the sixteenth century when an Italian naturalist, Aldrovandi, stated that copulation occurs among the snails. Although Wagner ('35, p. 304) had already indicated the fact that both sperms and eggs are derived from a single gonad and pass through a common duct before reaching the male and female conduits, most of the descriptions of the reproductive system of hermaphroditic gastropods published before the latter half of the nineteenth century fail to recognize the true nature of the ovotestis. Even Cuvier ('46) states that *Lymnæa* and *Planorbis* have the ovary and testis separate.

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

Among the most recent investigations of parthenogenesis in Gastropoda may be mentioned the work of Pelseneer ('19) on three species of *Lymnæa* and that of Robson ('23, '26) on *Paludestrina jenkinsi.* Pelseneer claims that eggs from his isolated snails gave off only one polar body. Colton ('18) found that the eggs of isolated *Lymnæa columella* regularly give off two polar bodies.

The most important cytological work on reproduction in pond snails is that of Colton ('18) in which he states that he found ripe sperms and ova in a single acinus in the ovotestis of *Lymnæa columella.* Diver ('25) deplores the lack of cytological investigation of the problem. Since neither the presence of two polar bodies in these eggs nor the fact that ripe sperms and ova occur in a single acinus at the same time does not preclude the possibility of parthenogenesis, these investigators have not solved the problem of reproduction in hermaphroditic snails.

In most cases the conclusions appear to be based upon scant evidence. For instance, only one case (Baltzer, '13) has been found in which the results obtained by isolation or breeding experiments have been substantiated by further cytological evidence than has already been mentioned. Although an enormous amount of classical embryological work has been done on gastropods, beginning with the efforts of Laurent ('37) and others, and extending to the last few years, and a considerable amount of excellent work has been done on fertilization in normal or so-called cross-fertilized species, very little work has been done on reproduction in isolated individuals. Lillie and Just ('25, p. 218) state that ". . . pulmonates appear to reproduce exclusively by cross-fertilization. . . ." However, they point out that the work of Braun, Colton and Cook in obtaining fertile eggs from isolated parents indicates that self-fertilization does occur in this order of mollusks.

The results obtained by various investigators indicate that the solution of the problem of fertilization in pond snails will be found by careful cytological studies on the eggs and oöcytes of isolated stock.

I wish to take this opportunity to thank Professor A. Franklin Shull for having directed this work. I am also indebted to the Graduate School of the University of Michigan for help from its research fund, and to staff members of the Departments of Zoölogy and Botany for favors, to the Marine Biological Laboratory for library and other facilities, and to several investigators at this laboratory and others in the Zoölogical Laboratory of the University of Pennsylvania for suggestions drawn from their various fields of specialization.

I. Material and Methods.

A. Material.

Individuals of the parent stock from which the different species used in these experiments were obtained were identified by Mr. W. J. Clench and these identifications were later verified by Doctor Bryant Walker. The nomenclature used in referring to North American forms is that of Walker ('12). In every instance "virgin" snails have been used, unless otherwise stated in the explanations. This "virgin" material was obtained by isolating an embryo and allowing it to hatch in a clear glass finger-bowl in which it remained until used or set free. Thus the eggs and ovotestis tissue from these "virgins" comprise the material upon which the results of this investigation are based.

The tissue and eggs of *Lymnæa stagnalis appressa* Say were used for this article, but the living and prepared eggs as well as sectioned tissue of virgin *L. columella, L. palustris, Physa gyrina,* and *P. sayii* were studied. *Planorbis trivolvis* were reared in strict isolation but did not lay eggs.

B. Methods.

a. Fixation.—The ovotestis of *L. s. appressa* is easily fixed in any kind of nuclear fixing solution with sufficiently good results to permit the study of germ cells in a general way though further study requires special technique. Eggs which have been oviposited present difficulties that are not overcome by any ordinary technique.

In general, it was found that a modification of Flemming's solution containing 2 cc. of 2 per cent. osmic acid, added at the time of use to 15 cc. of a solution consisting of 50 cc. of 1 per cent. chromic and 1 cc. of glacial acetic acid gave good results on both eggs and ovotestis tissue. Burkhardt's solution of potassium bichromate, chromic and acetic acids (*La Cellule,* 1897, p. 335), to each cubic centimeter of which about one half grain of cane sugar or sodium chloride is added, gives fair chromosomes with little cytoplasmic disturbance. This solution was used cold and at 30° to 85° C. When thus used, it thoroughly penetrates a whole specimen of *L. s. appressa* in a maximum of one hour or a mass of eggs in about two hours. A few minutes is sufficient for the actual penetration of the eggs; the additional time facilitates infiltration. As may be readily inferred tissue may be left in the above modification of Flemming much longer than in this modification of Burkhardt without seriously injuring it.

Bouin's picro-acetic and Allen's chromic acid and urea modification gave fair chromatin, but poor cytoplasmic fixation of tissue; however, these and other picric combinations were found to be of little or no value for eggs. It was found, as had Rockling ('22), on *Helix,* and H. Hoffman ('22) on *Limax,* that Zenker's fluid is a dependable fixing agent for general use. Very distinct mitotic figures and sperm asters were secured in ova which had been recently ovulated by using this agent at 50° C. However, it was useless for fixing eggs.

b. Imbedding.—In infiltrating and imbedding eggs and tissue, a number of aqueous media, several celloidin methods and John-

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ston's rubber-asphalt-paraffin, solutions of xylol and paraffin, chloroform and paraffin, etc., followed by pure paraffin, and pure paraffin alone were tried with varying degrees of success. It later became evident that most of the standard methods of infiltrating and imbedding tissues will give good results, if the tissue has been properly prepared to receive the medium.

The greatest difficulty encountered in imbedding the ovotestis may be properly charged to the cavernous liver and its surrounding membrane. Once these structures are freed of the clearing agent, infiltration is certain and complete if the tissue is left in the medium sufficiently long. These difficulties may be overcome by cutting the ovotestis into small pieces.

It is very difficult to obtain desirable serial sections of eggs which have been oviposited unless the albumen is properly treated before dehydration and infiltration are attempted. After the vitellus has been successfully fixed, enough of the albumen must be removed to permit the best infiltration of both the vitellus and the remaining albumen. Good serial sections of fertilization figures were obtained by treating previously fixed whole eggs with the following solutions: 2 per cent. potassium bichromate 100 cc., 0.5 per cent. chromic acid 100 cc., and nitric acid (C.P.) 6 cc. After this treatment the egg membrane and much of the albumen were cut away with a very sharp instrument made from a piece of a safety razor blade. Later, the vitelli were simply blocked out in this manner and the blocks imbedded without previous treatment in the above solution. Neither of these methods is favorable for the study of polar bodies and the former is the less favorable for the study of chromatin.

c. Staining.—The difficulties in staining and studying the slides are the result of the affinity of the polymorphic yolk granules for nuclear stains. After using a number of stains, Heidenhain's iron hæmatoxylin was adopted, alone or with Gage's acid fuchsin, lichtgrün, eosin or some other counterstain. Benda's crystal violet, gentian violet, Delafield's hæmatoxylin and saffranin were used with indifferent success.

II. COPULATION.

Pond snails, especially *L. s. appressa*, copulate freely throughout the year in laboratory cultures. On three different occasions

I have observed three individuals copulating with one another at one time, the foremost one functioning as a female, the middle one as both male and female, and the third as a male. Lankester ('74) and Baudelot ('63) cite instances in which several *L. stagnalis* were observed copulating "in chains," and Doctors Walker and Ruthven have observed the same thing in our wild snails. From the results obtained by rearing snails in isolation it would seem that copulation is unnecessary to perpetuate the species, unless, as some writers claim, self-copulation occurs. It has been suggested (Baudelot, '63) that copulation is necesssary to cause the ova to move out of the hermaphrodite gland and through the female conduit. However, most of the investigators who argue in favor of copulation claim that the sperms must first pass into the sperm receptacle where it is assumed apparently that they go through a process of ripening or are endowed with some peculiar properties before they can inseminate the eggs (Pelseneer, '96; Lacaze-Duthiers, '99; Baudelot, '63; Lereboulet, '62; Moquin-Tandon '55, and others).

Many writers cite Von Baer, 1835, as having observed a *Lymnæa auricularius* self-copulating (Braun, '88; Diver, '25), and Klotz ('89) states that Von Karsch observed an acting female while in copulation stretch its penis above itself and fertilize the acting male. Klotz ('89) observed simultaneous reciprocal copulation take place three times in wild and once in captive *L. stagnalis*. Kunkel observed *Arion* self-copulating (Robson, '23, p. 71). I have never observed actual self-copulation in any of my stock, although I have often seen apparent attempts at self-copulation in *L. s. appressa*. These attempts were always ineffectual so far as I was able to determine. In several cases the isolated individual might be seen at any hour of the day, and sometimes at night, with the copulatory organ evaginated and turgid, and on several occasions the groping male organ almost came in contact with the female orifice. In one instance I observed two *L. s. appressa* with ventral sides appressed, anterior ends reversed, apparently attempting to copulate reciprocally.

III. Oviposition and Viability of Eggs.

Wild snails lay only irregularly during the spring, summer and fall months, while my isolated laboratory stock lays about every third day for a period of about twenty-five days, rests a few days, then begins another cycle of laying. This is continued throughout the year. Of fifty-four *L. s. appressa* isolated from the same egg mass, seven laid their first eggs fifty-eight days after hatching. However, they do not reach their maximum laying capacity until they are about one hundred days old. The first mass is usually small, containing not more than twenty to twenty-five eggs. However, I have recorded instances in which the first mass contained two or three times this number. Kunkel states ('08) that two *L. stagnalis* deposited twenty-eight and thirty masses, respectively, in about fifteen months. Another individual deposited one hundred and sixty-eight masses in thirteen months (Holzfuss, '14). My snails were more prolific than this. An adult wild *L. s. appressa* was isolated in a two-gallon aquarium and given the best attention possible, that is, the water was never permitted to become the least stagnant, fresh leaf lettuce was always available for food and a green cabbage leaf was kept in water for her to oviposit on. She laid seventy masses, averaging between eighty and one hundred eggs each between November eleventh and the following June first. Most of these eggs were incubated until the embryo developed a shell. Thus it was found that at least 95 per cent. of the eggs of this isolated individual were viable. Similar tests were made with the eggs of a number of virgin *Physa sayii* and *L. s. appressa* with about the same results. Although no actual experiments of this nature were made with virgin *L. columella* and *L. palustris* eggs, casual observations indicate that the results would be comparable to those obtained for *L. s. appressa* and *Physa sayii.*

Planorbis trivolvis appears to offer an exception. Four individuals were isolated *in ovo* and carefully reared in isolation 377 days without their laying a single egg. The four snails deposited ten to fifteen small empty egg-mass cases during this time. Likewise no eggs were deposited by the control culture consisting of two individuals in a fingerbowl. My experience indicates that in all the pond snails studied, except *Planorbis trivolvis,* "virgins," *i.e.,* individuals isolated *in ovo,* lay as many eggs as individuals in mass cultures, and that the percentage of viability of these virgin eggs is as high as that of those from snails reared in mass cultures.

IV. SELF-FERTILIZATION AND PARTHENOGENESIS.

A number of investigators have isolated pond snails more or less carefully, and since they secured offspring they concluded that their snails reproduced parthenogenetically or by self-fertilization. Some investigators have tried to discriminate between self-copulation and internal fertilization, the latter being insemination that occurs in the hermaphrodite apparatus or uterus without self-copulation. Kunkel ('08) thinks that both internal self-fertilization and self-copulation occurs in *L. stagnalis*. Braun ('88) reared *L. auricularia* in isolation, and these produced offspring. Although he is not certain that self-copulation occurred in every case, he believes that self-fertilization took place. Colton ('22) self-fertilized *L. columella* for forty-seven generations. A. Lang ('00) thinks that self-fertilization without self-copulation occurs in *Lymnæa;* while Semper (Braun, '88) thinks that in all cases of reproduction in isolation self-copulation takes place.

The only investigators claiming parthenogenesis for fresh-water snails appear to be Robson ('23, '26) for *Paludestrina jenkinsi* and Pelseneer ('19) for *Lymnæa*. The latter states that in isolation cultures the eggs of *L. auricularia, L. glutinosus* and *L. palustris* give off only one polar body.

In my *L. palustris* isolated individuals lay eggs which extrude two polar bodies regularly. Colton ('18) states that in the eggs of isolated *L. columella,* two polar bodies are normally given off, and Kunkel (Diver, '25, p. 125) shows that two polar bodies are extruded in the eggs of isolated *Arion* and *Limax*. Thus Colton believes that isolated pond snails self-fertilize, while Pelseneer thinks that they reproduce parthenogenetically in isolation and also when mated with another species. In the latter case Pelseneer believes that the sperms from an individual of another species merely stimulate the egg to develop parthenogenetically, as Bělař ('24) has described in two forms of the nematode *Rhabdites*. Elswhere (Crabb, '27) the writer has pointed out that breeding experiments indicate that self-fertilization is the normal method of reproduction in pond snails.

V. PROTANDRY.

The question whether *L. s. appressa* is protandrous or protogynous has been raised. I have sectioned several young snails without finding definitive sperms or ova at any age up to thirty-five days. Pelseneer ('96) thinks that protandry should be considered general in the Euthyneura, especially in Pulmonata. Schapiro ('02) believes that parthenogenesis developed first in the animal kingdom, because it is more simple than hermaphroditism. On the other hand Brock ('86) thinks that the sex organs of Pulmonata are first laid down as female, and later become hermaphroditic. Hoffman ('22) found that the genital apparatus is not fully developed in *Limax maximus*, having a length of three centimeters. Certain Prosobranch gastropods have been shown to be protandric, for example, *Crepidula plana* (Gould, '17, '19), *Colyptræa sinensis, Crepidula unguiformis* and *Capulus hungaricus* (Giese, '15). Lams ('07) states that *Arion empiricorum* is protandrous. Ancel ('02) and Buresch ('12) appear to consider *Helix pomatia* and *H. arbustorum* as being neither protandrous nor protogynous, but hermaphroditic from the first differentiation of germ-cells. Since I examined sections of several young snails without finding definitive sperms or eggs at any age up to thirty-five days, I think that further work will show that *L. s. appressa* is neither protandrous nor protogynous, but strictly hermaphroditic.

VI. Development and Migration of the Germ-cells.

Both male and female germ-cells, as well as Sertoli and egg-nurse-cells, appear to be able to arise simultaneously from indifferent epithelial substance, and are commonly found developing together in a single acinus in *Lymnæ stagnalis appressa, L. columella* and *L. palustris*. Recent investigators are pretty well agreed that the origin of the germ-cells in most of the gastropods, especially in the Basommatophora and Stylommatophora is from indifferent epithelial material. Among these may be mentioned Garnalt ('88) who studied several forms of *Helix;* Buresch ('12) who worked on *Helix arbustorum;* Lams ('07) on *Arion empiricorum; and* Gatenby ('18) on *Helix aspersa, Testacella haliotiodes, Paludina, Lymnæa stagnalis* and other forms of *Helix* of uncertain taxonomic status. However, in his Text-fig. 5 and his text, p. 601 ('17), he appears to advocate a sort of "de-differentiation" idea for the origin of the germ- and nurse-cells in *Helix* *aspera.* He says: "Finally, I believe that the nucleus of the indifferent germinal epithelial cell may be stimulated by a variety of external agencies to tend towards one sex." His general opinion with regard to the condition in *Helix* in later papers (especially '19, p. 431) shows further that he believes that many germ-nurse-cells de-differentiate to form spermatocytes. As a result of further investigation on this subject ('22) he says: "The reason for the passage of the indifferent epithelial cell, either to the oögonium or spermatogonium, is at present unknown. Nutrimental conditions probably do not represent the real causal state." Buresch ('12) is satisfied that no cells ever de-differentiate. The work of Merton ('24) on the amœboid movements of ciliated-, nurse- and germ-cells and the phagocytic propensities of egg cells in *Planorbis* may indicate a way leading to the solution of this problem. Whether the germ-cells are derived from "indifferent epithelial substance" or from a "syncytium" or are "de-differentiated" from indifferent epithelial cells, or agents which cause early germ-cells to develop into ova instead of spermatozoa or *vice versa* is a question not within the scope of this paper.

The germ-cells continue to develop side by side until they become detached from the wall of the lumen. After this they remain associated until they reach the anterior part of the hermaphrodite duct where it is possible that their relative numbers may be reduced by a sort of segregation of the sperms and eggs shortly before reaching the oviduct and vas efferens. From the fact that it has been shown that in most species of hermaphroditic land and fresh-water snails the germ-cells develop side by side in the acinus, we know that this is not peculiar to *Lymnæa.*

The migration of the germ-cells is erratic inasmuch as it is begun in different stages of development in individuals of the same kind. For instance, some of the advanced eupyrene spermatids loosen themselves from the Sertoli cells, speedily ripen and thus begin the migration free in company with other spermatids, while in many other instances the Sertoli cells break loose from Ancel's layer and begin the migration with scores of normal advanced spermatids attached. In most instances these spermatids drop off before the nurse-cell reaches the hermaphrodite duct proper. At this time these spermatids appear as in Fig. 8. Frequently masses of sperms pass into the female conduit and are enveloped by the accessory coats of the egg along with the vitellus.

Whether the ovarian egg actually ruptures its follicle wall or merely absorbs it is not evident. However, since I have never observed a ruptured follicle, but have occasionally found free ova with a portion of the follicle wall still attached, I believe that instead of rupturing its follicle the ovum resorbs it, as Garnault ('89) describes in *Helix aspersa*. Henschen ('04) describes a "Zellmembran" in ovarian eggs of *L. stagnalis* which is probably the same structure that is called a hyaline membrane in this paper, but since he describes only ovarian eggs, it is impossible to tell definitely whether the membrane is the same in each case. Perez ('89) examined a large number of *Helix* at varying intervals after copulation but he was never able to observe ova passing through the hermaphrodite duct.

The ovum "ruptures" its follicle wall and begins the migration free in company with a multitude of male germ-cells in all stages of development from early spermatid to ripe spermatozoa. In most instances the egg is either completely or partially enveloped by a thin hyaline membrane (Figs. 1, 46) which functions as a sort of pseudo-vitelline membrane until it is absorbed by the ovum, or disintegrates. The ovum is usually free of this membrane before it reaches the hermaphrodite duct. Further evidence that this membrane is related to the follicle and not to the ovum is shown by the fact that occasionally free ova are found in the lumen of an acinus surrounded by a membrane having one or more disintegrating nuclei adhering to it. The presence of the hyaline membrane apparently bears no relation to the egg nucleus, for some free eggs in which the membrane of the germinal vesicle is intact have this membrane wanting entirely, while in others its persists over almost the entire egg until the first maturation spindle has begun to form (Figs. 1, 6, 7, 12, 14 and 46). Mark ('81, p. 178) says that "the yolk is certainly not provided with a distinct membrane . . .; only a very thin shell of protoplasm occurs in *Limax* eggs." Wierzjeski ('06) was unable to find a vitelline membrane on eggs of *L. stagnalis* before cleavage stages were reached. He states that a definite vitelline membrane has been demonstrated in the eggs of the snail *Paludina* by Tönninges.

Moquin-Tandon ('55) was unable to find a vitelline membrane but does not indicate the species of snail he was studying. Other forms have been described as not possessing a definite vitelline membrane; thus it is not surprising to find this membrane wanting in the eggs of *Lymnæa.*

VII. Maturation of Virgin Eggs.

Since in the maturation of eggs laid by snails which were isolated *in ovo* no marked differences from several published descriptions of supposedly cross-fertilized eggs of this and other forms of *Lymnæa,* as well as those of pond snails in general (Conklin, '10; Gatenby, '19; Kostanecki, '97; Morgan, '10, and others) were found, most of my evidence will be directed to points of comparison with maturation in forms other than *L. s. appressa.* I have observed two maturation divisions in living virgin eggs of *L. s. appressa, L. palustris* and *Physa gyrina,* and Colton ('18) found that *L. columella* regularly gives off two polar bodies. On the other hand Pelseneer ('19, p. 1058) is confident that virgin eggs of *L. auricularis* and *L. glutinosus* as well as *L. palustris* give off only a single polar body which never divides and is visible up to the gastrula stage. I am certain that Pelseneer's observations are incorrect in this respect, for in comparing virgin with normal eggs in the genus *Limnæa* he makes no mention of having ever found the first polar body isolated in the albumen of the egg. I have found the first polar body completely separated from the vitellus in *L. palustris* while in *L. s. appressa* it generally migrates into the albumen frequently to a distance of 50 to 200 micra or more before the first cleavage furrow is visible (Figs. 34, 35), consequently it may be easily overlooked in either living eggs or in sections. Byrnes ('99, p. 207) found that in normal *Limax agrestis* the first polocyte separates from the egg and collapses. In general Pelseneer's description is that of the second polar body, therefore I believe that he mistook the second for the first polar body.

In most instances the first polocyte is given off in from two to two and one half hours after the eggs have been oviposited. However, exceptions to the rule are numerous in *L. s. appressa.* In several instances the mass of eggs was seized with forceps before it was entirely out of the snail's body, pulled out and immediately fixed. Sections of these eggs were found in various stages of the first maturation division. Further proof of irregularity of maturation is shown in two eggs which were found in advanced cleavage stages in the hermaphrodite duct of different snails (Figs. 17, 18). The maturation divisions come surprisingly near being synchronous in all the eggs of a given mass, the laggards being usually those at the ends of the mass. Kofoid ('95) found much variation in *Limax* eggs of the same mass.

Conklin ('10, p. 420) seems to have found a germinal vesicle regularly present in eggs of *L. columella* which had been oviposited, for he says: ". . . As the germinal vesicle begins to dissolve and the first maturation spindle appears, the clear area of the germinal vesicle becomes elliptical and then spindle-shaped in outline." It is evident he is using the term "germinal vesicle" to include all nuclear stages before the polar bodies are extruded, for a true germinal vesicle, as defined by Purkinje, 1825 (Wilson, '25) was not found in living or sectioned eggs laid by *L. s. appressa*. Since only one instance in which the germinal vesicle persisted until the egg reached the hermaphrodite duct has been found (Fig. 6) and since well-formed spindles occurred in several eggs within the hermaphrodite duct (Figs. 11, 12, 13, 15 and 16) I am of the opinion that in the eggs of *L. s. appressa* the germinal vesicle has disappeared by the time the egg enters the oviduct.

My observations on the living eggs of both "virgin" and "normal" *L. s appressa* are in accord with Conklin's work ('10) inasmuch as the clear area "becomes elliptical, then spindle-shaped, . . . then moves through the egg until one end of the elongated area comes into contact with the surface at the animal pole of the egg, leaving a deep 'well' of clear protoplasm leading down to the center of the egg." This "well" is the result of forces connected with mitosis by means of which the yolk granules are displaced, or almost so, thus leaving an apparently clear area in living eggs, the "well," in which the first maturation spindle is formed and subsequently migrates to the animal pole of the ovum (Figs. 49, 51). A clear area forms similarly in the egg of the worm *Nereis* (Lillie, '12); the echiuroid *Thalassema* (Griffin, '99) ; the nemertean *Cerebratulus* (Coe, '99) ; the prosobranch *Fasciolaria* (Hyman, '25) ; the gastropod *Limax* (Mark, '81) and in several other forms.

The first polocyte forms a protuberance (Fig. 51) which soon becomes a pedunculated body due to the constriction of the stalk, and at about this time yolk granules obliterate the clear area. The first polocyte may remain attached to the ovum, but more often it becomes entirely disconnected and often may have migrated into the surrounding albumen a distance equal to twice the diameter of the vitellus before the first cleavage occurs (Fig. 34). Although in this work hundreds of living eggs were observed undergoing maturation and cleavage and these stages studied in a great number of sectioned eggs, I have never found a case in which the cytoplasm of either polocyte had divided to form another body such as frequently occurs in molluscan and other eggs, and as is figured by Kostanecki and Wierzjeski ('96) in *Physa fontinalis.*

Within about fifteen minutes after the first polar body becomes pedunculated the animal pole is free of most of the yolk material thus giving this region a clear appearance; then the second polar body appears as a clear, slightly bulging region near the same point where the first was formed. It reaches its maximum proportions in one to two minutes more, and in a total of three to five minutes from the time the animal pole of the egg becomes clear the second time, the second polar body is completely formed, and the animal pole is again darkened by the return of the yolk materials. Thus the entire cycle resulting in the extrusion of the first and second polocytes is completed in about seventeen minutes. This schedule holds only in water having a temperature of about 20–22° C. A drop in the temperature of two to five degrees below twenty has a very noticeable retarding effect on the maturation of the egg. Kofoid ('95) found that temperature has a profound effect upon the eggs of *Limax.* Vignal ('11) observed that this is also true of *L. stagnalis* eggs. Brynes ('99, p. 207, 209) found that in *Limax agrestis* the first polocyte is formed and extruded in two minutes and the second is formed and becomes detached in the same length of time. In the living eggs of *L. s. appressa* the chromosomes of the first polocyte may often be seen arranging themselves into an equatorial plate. Few or no yolk granules pass into either polocyte and one or both polocytes may remain attached to the egg for several cleavages, or even until the gastrula stage. Holmes ('00) found that in *Planorbis* the polar bodies were retained until the gastrula stage was reached.

Sections show that the chromosomes of the first polar body normally arrange themselves in an irregular equatorial plate, and in some instances definite monocentric mitoses, such as are shown in Figs. 34 and 59 occur. Griffin ('99) found that in the Echiuroid *Thalassema* the first polocyte normally divides synchronously with the formation of the second, the chromosomes forming double elements before dividing and in general behaving as do the egg chromosomes. He shows that rarely by the time the second polocyte was extruded the chromosomes of the first polocyte had completed mitosis to the telophase without the cytoplasm having divided.

In *L. s. appressa* the second polocyte forms about four distinct vesicles synchronously with the formation of the egg nucleus (Figs. 34, 40). These vesicles disappear some time before those of the egg fuse, leaving a few more or less deeply staining chromomers which become larger, more numerous and stain more deeply as the first cleavage nucleus is formed (Figs. 27, 32, 35, 42).

VIII. Insemination.

At first thought one would be led to assume that self-fertilization and polyspermy occur under such conditions as have been shown to exist in the hermaphrodite apparatus of *L. s. appressa* unless some barrier, such as Morgan ('04, '05, '23) found in *Ciona* existed. Investigation shows that this is the case; the hyaline membrane acting as a barrier to the entrance of the spermatozoa until an area of the egg is freed from it (Figs. 1, 12, 46).

A. Conoid and Round-head Spermatozoa.

Ova from the hermaphrodite duct contain only round-head sperms (Figs. 6, 15, 16) and are usually surrounded by the same type of spermatozoa (Fig. 10), while those ova from the acini of the ovotestis usually contain only conoid sperm heads (Figs.

2, 3, 4, 5) and are accompanied by spermatozoa of the same kind (Fig. 9). Since, from smear and sectioned preparations, it appears that the conoid spermatozoa are found more often than the round-headed kind in the upper part of the hermaphrodite duct of different individuals and since the latter have not been found in the acini, it appears that the two types are not different kinds of sperms, but merely represent different ages. Thus it is assumed that the round sperm heads found in ova from the hermaphrodite duct were conoid when they entered the ova, probably while the latter were high up in the acini. The heads then began to swell, lost their tails and by continued swelling assumed spherical forms (Figs. 6, 19, 21, 25) and finally disintegrated; the nuclus of one of them normally becoming reorganized to form a vesiculated and later a definite pronucleus (Figs. 26–41). Among sperms which have never entered an ovum I have traced stages of metamorphosis paralleling those which occur within the egg, except that spermatozoa within the sperm receptacle form a pronucleus which does not possess karyomeres (Fig. 44). The dumb-bell-shaped sperm heads which occur among ova in the hermaphrodite duct are probably fused spermatozoa such as Retzius (Wilson, '25, p. 305) describes for the gastropod *Turritella*.

That these round-head sperms are not the result of faulty fixation is shown by the fact that they are regularly obtained with Zenker, Perenyi and modified Flemming solutions. In view of the fixations used the number and definiteness of these structures in the ova and among the spermatozoa practically precludes the possibility of their being cell inclusions. Neither should these be classed with the "apyrene, oligopyrene, hyper-pyrene" and other similarly abnormal sperms such as Gatenby ('16) describes for *L. stagnalis*, Ankel ('24) for *Bythinia* and others for other forms, for these abnormalities are not sufficiently numerous to play any part in the relative numbers of conoid and round-head individuals.

B. Ovarian Insemination.

Although I have not been able to show conclusively that ovarian ova are sometimes inseminated in pond snails, I have seen instances, especially in *L. columella,* in which ripe spermatozoa appear to have penetrated ovarian ova. Some additional evidence of ovarian insemination is shown in Fig. 12, in which an ovum that has not yet reached the hermaphrodite duct proper contains four sperm heads and a body that is probably a degenerating or abortive sperm pronucleus. The location of this ovum and the presence of a hyaline membrane over a considerable area of its surface indicate that it has only recently left its follicle, while the forming archiamphiaster and the round-head sperms argue for an earlier insemination than would normally occur in an egg in this region of the ovotestis. Fig. 14 shows another ovum which similarly indicates ovarian insemination.

The presence of spermatozoa in ovarian ova has been described by Buchner ('14, '15) in the Archiannelid *Saccocirrus* and in one of the Turbellaria, and by Nachtsheim ('19) in *Dinophilus apatris*. Lams ('07) found in the slug *Arion empiricorum* that "fecundation was often intraovarine" and that segmentation stages, including the morula and blastula, occur in the interior of the ovotestis. "The mass of blastomeres was entirely surrounded by follicle cells. One is able therefore to say that in *Arion* the development sometimes begins in the ovary."

C. Normal Insemination.

From my material of *L. s. appressa* it is evident that the earliest insemination normally takes place within the acinus in which the ovum and the spermatozoa which enter the ovum are developed. Additional sperms continue to enter the ovum until it passes out of the hermaphrodite duct, but it is clear that one and only one sperm pronucleus fuses with the egg pronucleus. The supernumerary sperms normally disintegrate, or possibly pass through the ovum before the egg pronucleus is formed (Figs. 14, 32).

Since the yolk granules interfered with studying the sperms, several living adult *L. s. appressa* were centrifuged and immediately fixed in a warm fixing fluid. In one of the snails there were five free ova in the acini. In two of these which had very recently lost their hyaline membranes, marked cytoplasmic disturbances resulted from centrifuging them. The periphery of each ovum suffered severe fragmentation and dissociation of the yolk granules. Although much cytoplasm remained around the germinal vesicle the yolk granules were sufficiently displaced to make it easy to find the enclosed conoid sperm heads and to follow the course of their tails (Figs. 2–5).

D. Changes in the Oöcyte Following Insemination.

Whether or not there is a volumetric change following insemination in *L. s. appressa* eggs, such as has been found in the case of the brook lamprey (Okkelberg, '14) and in the echinoderms *Arbacia* and *Asterias* (Glaser, '14) and *Asterina* (Snyder, '25), is not evident. However, ova were found which appear to indicate such a change. The sections of the smallest of these abnormal ova, from near the anterior end of the hermaphrodite duct, were about one fourth the diameter and number of those of individual ripe ovarian and recently "ovulated" ova.

E. Formation of the Sperm Amphiaster.

The early sperm aster occurs only in ova which have not undergone the first maturation division, and may be readily recognized by its great size, which nearly equals that of the early egg asters. This primitive sperm aster, or archiaster, is characterized by having long stout rays and indifferently formed centrosomes (Figs. 11, 13, 15, 16). Perhaps these should be considered as unusual centrospheres instead of centrosomes. It is apparent that the sperm archiaster normally undergoes a sort of metamorphosis before it forms a typical amphiaster. During this period of change there is a marked reduction in the length of the rays and a great condensation of the chromophilic substance in the centrosome. The *modus operandi* of this metamorphosis is obscured by the large number of polymorphic basophilik yolk granules which retain the stain as readily as the centrosomes themselves. My material indicates that during the prophases of the first maturation division of the oöcyte, these sperm archiasters reach their maximum size and subsequently the typical aster reforms from the granules of the disintegrated centrosome of the archiaster and reappears as a very small aster having short delicate rays and a very condensed deep-staining centrosome. The amphiaster most commonly arises from this aster (Fig. 23), about the time of the first maturation of the egg (Fig. 20–25) but may arise earlier (Figs. 19, 21).

A novel opportunity to study the amphiaster presented itself in an ovum which had been deposited in the sperm receptacle of a snail functioning as a female during copulation (Fig. 19). In this ovum the centrosome of the archiaster has presumably been reduced to the typical aster-stage and is now divided to form an amphiaster in which each centrosome is composed of three distinct centrioles similar to Fig. 25 which is a later stage and shows numerous centrioles associated with the vesiculated sperm head. The sperm tail is distinctly visible but is not connected with the amphiaster; the distal member of the archi-amphiaster is at the periphery of the ovum. The presence of six centrioles in this sperm amphiaster (Fig. 19) and of several in a more nearly mature egg (Fig. 25) support the conclusions drawn from Figs. 11, 12, 13, 15 and 16, and other material fixed with Zenker's fluid, which does not give such fine detail as the modification of Flemming's mixture with which this unusual egg and that represented in Fig. 25 were killed. This is in general agreement with the work of Kostanecki and Wierzejski ('96) and Wierzejski ('06) on "cross-fertilized" eggs of *Physa fontinalis* with the exception that they figure a single centriole in each centrosome.

Mark ('81) described in *Limax* a difference in the size and form of the centrosomes and astral rays of the egg "archi-amphiaster" of Whitney as compared with the cleavage amphiasters which parallel the relation of the sperm archiaster to the sperm amphiaster which I have described for *L. s. appressa*. He figures and describes a single "abnormal" egg having six "large supernumerary asters with large centrosomes and very stout rays" (Fig. 81 and p. 221). A similar metamorphosis of the egg archi-amphiaster and of the sperm archiaster has been demonstrated by Foot and Strobell ('00, Fig. 9) for the worm *Allolobophora fœtida*.

A typical sperm aster has not been visible in virgin eggs until the first polar globule has been extruded; however, vesiculated sperm heads occur in younger eggs (Fig. 21). In the ovum shown in Fig. 14 the degenerating amphiasters must be those of supernumerary sperms, while all traces of the functional sperm are masked by yolk granules. Pieces of swollen, deeply stained sperm tails were found in ova sectioned in the acini, but no asters occurred with them. Figs. 20–25 show several stages in the formation of the sperm amphiaster in virgin eggs. Fig. 25 represents an early stage in the formation of the vesiculated sperm pronucleus and is the only one of its kind found.

Kostanecki and Wierzejski ('96) found that in eggs of *Physa fontinalis* which had been reared in mass cultures the sperm amphiaster is often formed before the anaphase of the first maturation division (Taf. XVIII., 3, 4) and that the sperm pronucleus may have formed before the second polar body is given off (ibid., 4). Byrnes ('99, p. 215) states that in *Limax agrestis* the "relations of the sperm head, the asters and the maturation spindle are precisely similar to those figured by Kostanecki and Wierzejski for *Physa*." Thus the work of Kostanecki and Wierzejski on *Physa* eggs, that of Byrnes on *Limax* eggs as well as my work on virgin *L. s. appressa* eggs shows that there is no real relation between the maturation activities of the egg and any stage in the development of the sperm amphiaster or pronucleus. Rather, it appears that the state of the development of the sperm pronucleus is related to the time at which the selected sperm entered the ovum. The work of these investigators indicates that the process of fertilization in their "normal" *Limax* and *Physa* eggs is quite similar to that in my "virgin" eggs.

F. Formation and Fusion of the Pronuclei.

Male Pronucleus.—After the head of the spermatozoön has reached its maximum size it loses its ability to take basic stains and appears as a hyaline area or vesicle in the egg cytoplasm (Figs. 19, 21, 25). Although there appears to be no connection between them, the sperm amphiaster is associated with the vesiculated head. Kostanecki and Wierzejski ('96) show several figures of *Physa* eggs in which the amphiaster has migrated a relatively great distance from the sperm head before the latter formed a pronucleus. In my material the sperm pronucleus becomes vesiculated about the time the second polocyte and the egg pronucleus are formed of definite karyomeres.

Female Pronucleus.—Following the telophase of the second maturation division, the egg chromosomes form vesicles which appear to be more or less independent of each other. Although with few exceptions they are tangent at the first (Fig. 37), they soon become somewhat appressed as in Fig. 29. As the sperm pronucleus comes in contact with the egg nucleus, the karyomeres of each nucleus come to lie closer and closer together so that by the time the nuclei have become appressed the number of chromosome vesicles in each has been reduced by fusion. This fusion continues so that the identity of each pronucleus is lost (Figs. 35, 39). Ultimately all the egg chromosome vesicles fuse to form a typical pronucleus surrounded by a distinct and apparently continuous membrane. The definitive sperm pronucleus is formed at the same time and in the same manner (Figs. 39, 41). Chromomeres are abundant in both pronuclei but appear to be separate rather than " strung on a linin thread " in resting nuclei as well as in fusing pronuclei (Figs. 41–43). Thus the formation of the pronuclei and of the first cleavage nucleus in *L. s. appressa* is essentially parallel with that in the worms, *Eustylochus* (Planarian), (Van Name, '99), *Dinophilus* (Nachtsheim, '19),*Chætopterus,* (Mead, '95), *Nereis* (Lillie, '12), *Platynereis* (Just, '15), and Amphitrite (Scott, '06) ; an echiuroid, *Thalassema* (Griffin, '99) ; the hymenoptera *Acrochismus* and the bug *Icerya* (S. H. Schrader, '24, '25) ; the mite *Tetranychus* (F. Schrader, '23) ; the daphnid *Polyphemus* (Buchner, '15, citing Kuhn) ; the nudibranchs *Doris* and *Montagua* (Smallwood '05), and other forms.

Kostanecki and Wierzejski ('96) figure chromosome vesicles which appear as lobules on both pronuclei (Taf. XX., Figs. 25, 28). The absence of definite chromomeres in their pronuclei possibly indicates that little attention was given to staining and studying the nuclear substances. The fusing pronuclei lose this lobulated appearance and have definite nuclear membranes (Taf. XX., Figs. 30–34) as is the case in those forms which are known to form the egg pronucleus from karyomeres. Mark's figures ('81) suggest a vesiculated stage in the pronuclei of *Limax campestris,* but he does not recognize such a condition in his text description. Griffin ('99) shows a distinctly vesiculated egg nucleus in *Thalassema* (Fig. 29) and vesiculated individual chromosomes in the telophase of the second maturation division (Fig. 55).

IX. THE CHROMOSOMES.

The chromosome number has been established for a number of the land gastropods. However, practically nothing definite is known about the chromosome number in hermaphroditic fresh-water snails. Harvey ('20) citing Linville, 1900, lists sixteen chromosomes each for the primary and the secondary oöcytes of *Lymnæa elodea.* The chromosomes of *L. s. appressa* are very irregular in shape and apparently in number, and this numerical variation appears to be the result of splitting of chromosomes (Figs. 55, 58, 59).

A. In Egg Cells.

Because of the great number of irregular yolk granules in the egg cytoplasm which take up the basic stains more readily and retain them more tenaciously than the chromatin itself does at times, it is difficult to distinguish the chromosomes clearly, especially in stages other than the early equatorial. After the germinal vesicle breaks down there is no definitely visible egg chromatin until a number of polymorphic granules appear among the spindle fibers of the archiamphiaster (Figs. 11, 12, 13, 16). The various stages in the history of the development of the egg nucleus between the formation of the archiamphiaster and the extrusion of the first polocyte are not sufficiently well known to warrant discussion at this time. However, it appears that late prophase stages are formed as indicated in Figs. 48, 49 and 50.

a. First Polocyte.—Ten chromosomes are readily distinguished in most of the recently extruded first polar bodies, but this number is rapidly increased as the second maturation division of the egg progresses so that by the time the second polocyte is cast out of the egg the first may have fifteen to twenty chromosomes in it. Fig. 53 represents the first polocyte of an egg in which the second polar body is shown forming in Fig. 60. Each of the three first polar bodies represented in Fig. 52–54, as well as the egg nucleus in Fig. 52, contains ten chromosomes. In Fig. 55 four of the ten chromosomes have split, thus making the first polocyte seem to have fourteen chromosomes. Likewise the chromosome number of the first polocytes represented by Figs. 56 and 57 appears to be about thirteen. The latter was fixed during the metaphase of the second maturation and the former after the second polocyte had been extruded. Fig. 58 shows a first polocyte of undetermined age in which six chromosomes appear as pairs and others have probably been divided so long that the halves are now completely separated, thus bringing the apparent number up to about eighteen. Whether or not this high number of chromosomes is due to monaster activity is not known. However, a first polocyte which has undergone monocentric division is shown in Fig. 59, and another is shown in monocentric metaphase in Fig. 34. The chromosome vesicles in the second polar body have disappeared and fusion of the egg karyomeres is nearly complete in Fig. 35, while in Fig. 34 these conditions are not so far advanced.

The foregoing observations indicate that there is an actual time relationship between the age of the first polocyte and the apparent number of chromosomes it contains and that this relation holds true until the pronuclei are formed. No further data on this point are available than the first cleavage nucleus (Fig. 42). However, the chromosomes in the first polocyte apparently disintegrate soon after having undergone monocentric mitosis and before the first cleavage.

b. Second Polocyte.—In Fig. 60 ten chromosomes are shown remaining in the egg during the second maturation anaphase. I am at a loss to explain the apparent number and pseudo-equatorial plate formation of the chromosomes which are being extruded in this instance. The pseudo-equatorial plate really lies more nearly on its edge than is shown in Fig. 60. Successive changes in the second maturation anaphases are shown in Figs. 61–63. In each case approximately ten chromosomes are destined to be extruded and as many to be left in the egg. The chromosomes left within the egg, as well as those within the second polar body, form vesicles containing chromomeres (Figs. 26–37, 40). The vesicles of the second polar body appear to lose their membranes and thus set the chromomers free within the cytoplasm of the polocyte (Figs. 35, 42).

c. Karyomeres.—The chromosome vesicles or "karyomeres" (Conklin, '02) of the egg nucleus, which form after the extrusion of the second polar body, offer some evidence for determining the haploid number of chromosomes which should be considered. Several investigators have shown conclusively that in many plants and animals the chromosomes form individual vesicles which in turn fuse to form the pronucleus or nucleus as the case may be. Richards ('17) has worked out the history of the chromosomal vesicles in *Fundulus,* and Nekrassoff ('04) has traced the metamorphosis of a single chromosome of the second maturation division from the anaphase through several stages to the formation of a large spherical vesicle, such as occurs in *L. s. appressa,* in *Cymbulia.* From this work it appears that the number of chromosome vesicles in the egg pronucleus is the same as the number of chromosomes. However, he does not attempt to correlate chromosome number with chromosome vesicle number in this or his later article ('09). More than ten karyomeres have not been found in mature eggs of *L. s. appressa.* However, various numbers from one, in which all the karyomeres are fused to form the definitive egg pronucleus (Fig. 41) to nine or ten (Figs. 29, 37), have been found. It is significant that the maximum number of these vesicles is the same, ten, as the number of chromosomes in the mature egg and in the first polocyte.

Other investigators have found that the maximum number of karyomeres in the mature egg is the same as the haploid chromosome number. Nachtsheim ('19) found this to be the case in *Dinophilus;* F. Schrader ('23) in *Tetranichus,* and S. H. Schrader ('24), in *Acrochismus.* No signs of karyomeres were found in male cells of *L. s. appressa* other than as shown in the pronuclei. In this connection it is interesting that the male pronuclei formed in the sperm receptacle are only of the type having a definite membrane (Fig. 44) and resembling those of fowl spermatozoa developed in culture media by Loeb and Bancroft ('12).

B. In Sperm Cells.

It appears that the haploid number of chromosomes in the sperm cells is about ten (Fig. 65). The writer hopes to be able to throw further light upon the chromosomes in spermatocytes, primary oöcytes and cleavage nuclei in a later paper.

The principal points in the chain of evidence which indicates that the haploid chromosome number in *L. s. appressa* is ten are:

(*a*) ten chromosomes is the usual number found in young first polocytes, and an equal number normally remains in the egg; (*b*) the anaphase of the second maturation division usually shows ten chromosomes at each pole and (*c*) the observed maximum number of karyomeres which go to form the definitive egg pronucleus is ten.

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X. Conclusions.

From the evidence brought out in this paper it appears that self-fertilization is the normal method of reproduction in *L. s. appressa* and that in this snail cross-fertilization seldom or never occurs. The reasons for concluding that self-fertilization is the normal method of reproduction are as follows:

1. Both ova and spermatozoa are developed in a single acinus at the same time, and since the ovum soon loses its investing membrane polyspermy usually occurs before it leaves the acinus.

2. At no time are functional sperms absent from the hermaphrodite gland and duct in normal healthy adults, thus by the laws of chance making competition of foreign sperms unsuccessful.

3. Free ova lacking a vitelline membrane are usually surrounded by ripe sperms, numbers of which enter each ovum as it passes through the acinus and hermaphrodite duct.

4. There is no evidence of desquamation of the lining in any part of the reproductive system, as has been described in Helix, or of any other natural process which would cause temporary or permanent, unisexuality in *L. s. appressa*.

5. Individuals raised from isolated eggs and reared in strict isolation reproduce as abundantly as do those in mass cultures.

6. There is no evidence of gynogenesis or any other form of parthenogenesis.

7. Two polar bodies are extruded in eggs of virgins, the first normally loses connection with the vitellus and by the time of the first cleavage has migrated 50–200 micra into the albumen; the second polocyte remains attached to the vitellus, but its chromatin does not return to the egg nucleus.

8. Ten chromosomes comprise the haploid number as is shown by the first and second maturation divisions and by the number of karyomeres in the mature egg.

9. Typical male and female pronuclei are formed, and fuse in virgin eggs to form the first cleavage nucleus.

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EXPLANATION OF PLATES.

The figures of all the plates have the same reduction and all, except Figs. 8, 9, 10, 17, 18, 44, 45 and 46, were outlined with the camera using 10X ocular and 1.9 objective (scale 0.03 mm., Pls. 4, 5) or 18x ocular and 1.9 objective (scale 0.02 mm., Pl. 5).

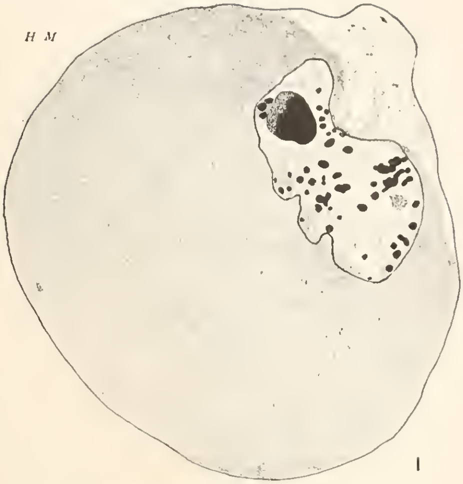
PLATE I.

Free ova from the acini of the ovotestis near the beginning of the free hermaphrodite duct (region *H*, Fig 1, Crabb, '27*b*), except Fig. 6, scale 0.03 mm.

FIG. 1. A recently ovulated egg completely enveloped by the hyaline membrane (*HM*); germinal vesicle intact. Burkhardt and cane sugar, 6 μ, iron-hæmatoxylin.

FIGS. 2, 3, 4, 5. Fragments of two centrifuged ova which have recently lost the hyaline membrane. The yolk granules have been dispersed and in addition the peripheral region is badly fragmented, thus making it an easy matter to trace the spermatozoa in the cytoplasm. Fig. 3 represents a section of a large peripheral fragment and shows numerous spermatozoa. The living snail was centrifuged 15 minutes and immediately killed in Burkhardt's solution and NaCl at 48° C., 6 μ, iron-hæmatoxylin.

FIG. 6. An inseminated egg from the hermaphrodite duct (*L* to *O*, Fig. 1, Crabb, 27*b*). Reconstructed from two non-consecutive sections. The germinal vesicle and round sperm heads without tails are from one section, while those having tails are from a peripheral section. Perenyi, 6 μ, iron-hæmatoxylin.



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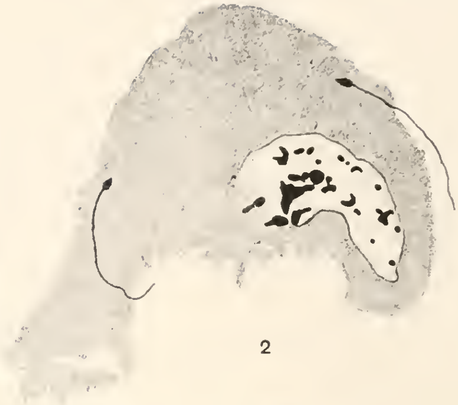

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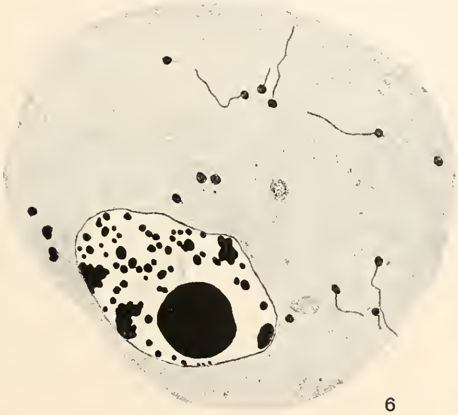

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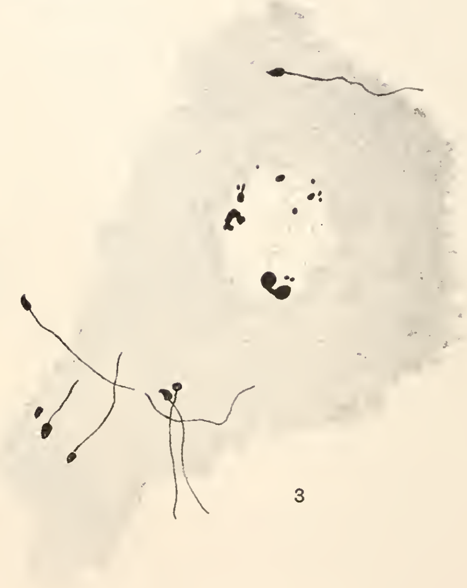

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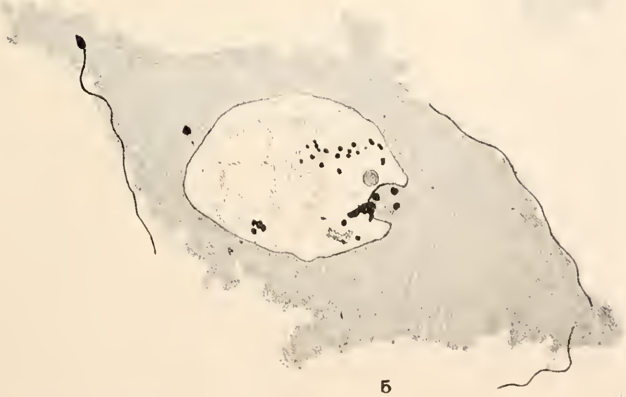

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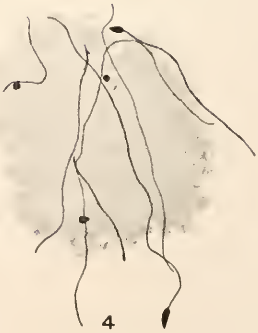

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EDWARD DRANE CRABB.

8

PLATE 2.

Free ova from the hermaphrodite apparatus of three different individuals. Figs. 11 and 13–16 are from the same snail. Scale, 0.03 mm., Pl. 4, except Figs. 8–10.

FIG. 7. A single section of an ovum, from an acinus, which is entirely free from the hyaline membrane and shows high polyspermy, Modified Flemming, 8 μ, iron-hæmatoxylin.

FIG. 8. A young sperm head which has just loosened its hold on a Sertoli cell. Much enlarged.

FIG. 9. A ripe conoid sperm head from an acinus. Much enlarged

FIG. 10. A round-head sperm from the hermaphrodite duct. Much enlarged.

FIG. 11. An ovum from the hermaphrodite duct, reconstructed from three sections; supernumerary sperm heads omitted. Zenker, 40° C., 7 μ, iron-hæmatoxylin, eosin.

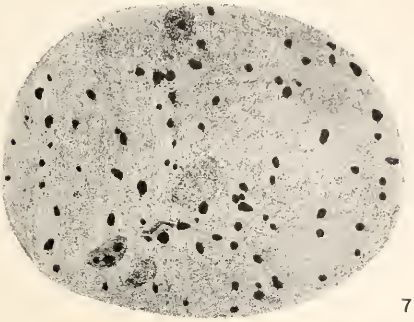
FIG. 12. A free ovum nearly surrounded by the hyaline membrane with forming first maturation spindle, three sperm heads and a structure which may be an abnormal sperm pronucleus and which was transposed from the next section. Modified Flemming, 8 μ, iron-hæmatoxylin.

FIG. 13. An ovum from the hermaphrodite duct, reconstructed from five sections which are consecutive except that one is missing. Zenker, 40° C., 7 μ, iron-hæmatoxylin.

FIG. 14. An ovum from the same slide and region as Fig. 7 showing degenerating (?) supernumerary sperm amphiasters and numerous large yolk granules. All structures, except the two central clear areas, are from one section.

FIG. 15. An ovum from the hermaphrodite duct, reconstructed from three consecutive sections showing a supernumerary aster (archiaster), early first maturation spindle ("archiamphiaster," Mark, '81), and several disintegrating sperm heads which occurred on a single section. Same slide and region as Fig. 11.

FIG. 16. An ovum from the hermaphrodite duct, reconstructed from six consecutive sections. Three sperm archiasters and seven heads are all from the same section. The archiamphiaster may be shown too long. Zenker, 40° C., 7 μ, iron-hæmatoxylin, eosin.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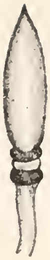
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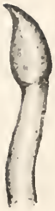
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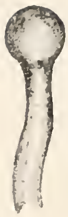
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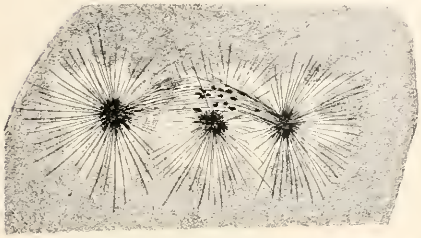
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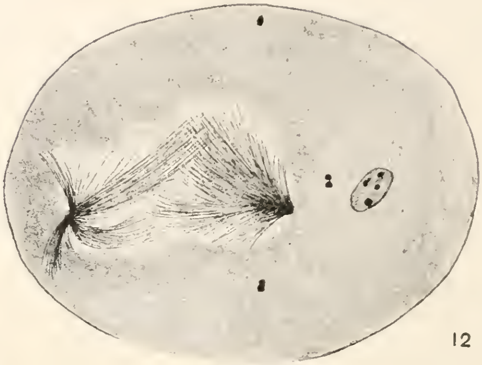
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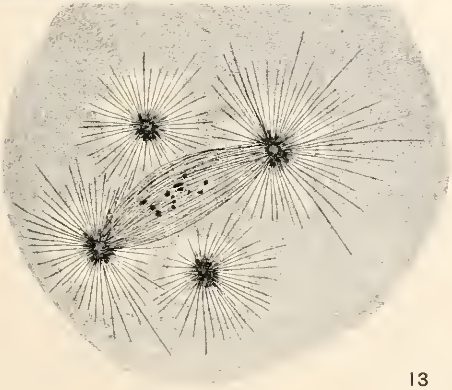
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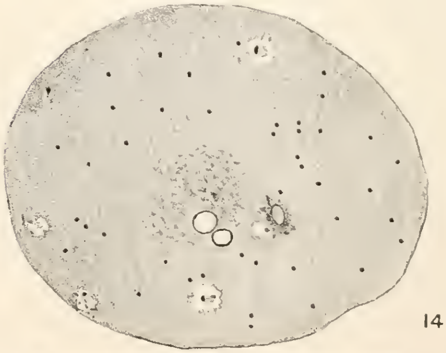
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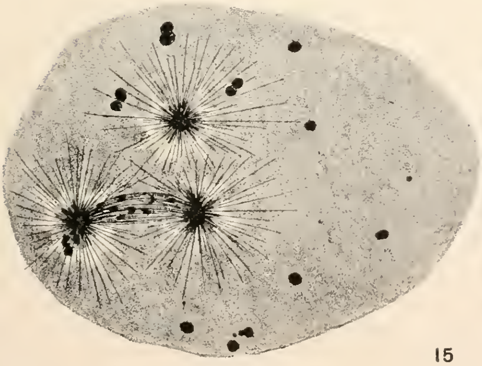
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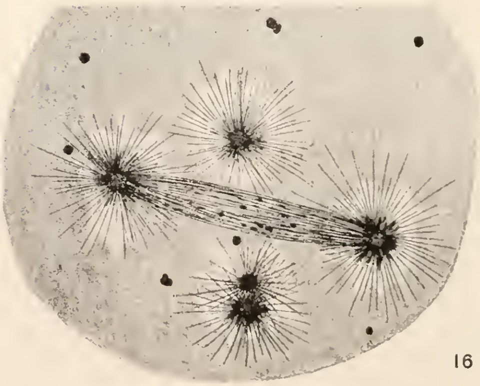
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EDWARD DRANE CRABB.

PLATE 3.

All figures except 19 are from virgin eggs. Figs. 19 to 25 were drawn to the scale of 0.03 mm., Pl. 4; Figs. 17, 18, to 0.04 mm.

FIG. 17. Four-cell stage from the hermaphrodite duct. Zenker 40° C., 55 minutes, 7 μ, iron-hæmatoxylin.

FIG. 18. A later cleavage stage from an acinus (region *H*, Fig. 1, Crabb, '27*b*). Modified Flemming, 8 μ, iron-hæmatoxylin.

FIG. 19. Reconstructed from two consecutive sections of an ovum deposited in the sperm receptacle during copulation. The piece of sperm tail and its vesiculated head are on one section, the archiamphiaster is on the other. Modified Flemming, 8 μ, iron-hæmatoxylin.

FIG. 20. Reconstructed from two consecutive sections of an egg before the second polocyte is extruded, showing the vesiculated sperm head and its aster. Modified Flemming, 10 μ, iron-hæmatoxylin, Gage's acid fuchsin.

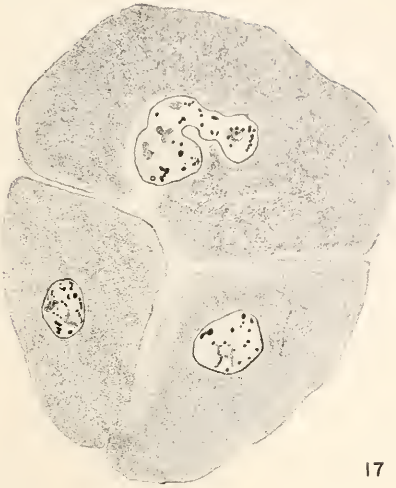
FIG. 21. Reconstructed from three consecutive sections of a freshly laid egg showing first maturation spindle, sperm head and tail. The sperm head is more vesiculated than is usually found in eggs after the first maturation division. The piece of sperm tail is not stained. No asters could be seen. Modified Flemming, nitric acid solution, 6 μ, iron-hæmatoxylin.

FIG. 22. Reconstructed from the first and fourth of four consecutive sections of an egg which has recently given off the first polocyte. The vesicle-like sperm head is at a lower level than its aster. Modified Flemming, nitric acid solution, 10 μ, iron-hæmatoxylin, Gage's acid Fuchsin.

FIG. 23. Reconstructed from three consecutive sections of an egg which has given off the first polar body, showing sperm amphiaster forming. Modified Flemming, 6 μ, iron-hæmatoxylin.

FIG. 24. An egg after the first maturation division showing the vesiculated sperm head and an aster. Fixed one hour and twenty minutes after being oviposited. Modified Flemming, 4 μ, iron-hæmatoxylin.

FIG. 25. An early stage in the formation of sperm karyomeres. The egg chromosomes are somewhat vesiculated, but the second polocyte has not been extruded. Modified Flemming, 4 μ, iron-hæmatoxylin.



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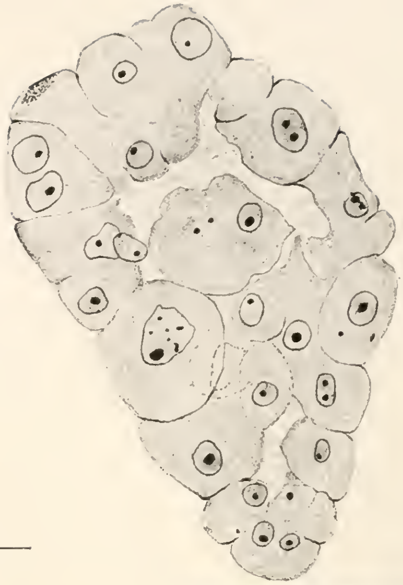

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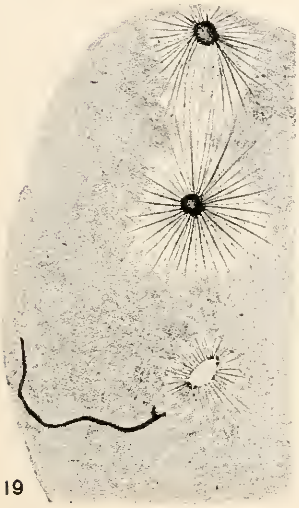

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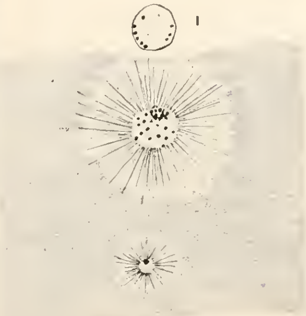

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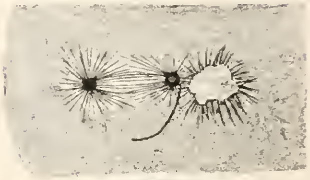

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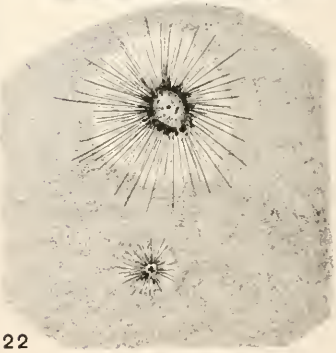

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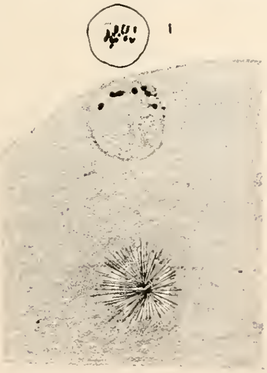

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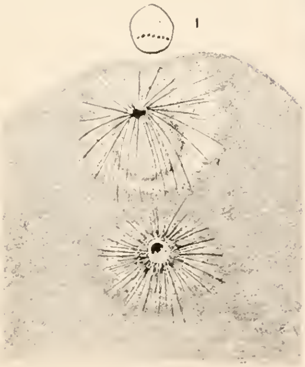

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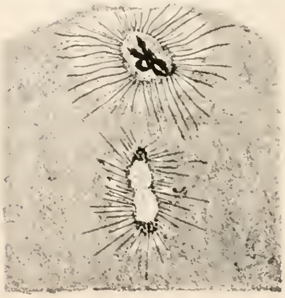

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EDWARD DRANE CRABB.

PLATE 4.

Virgin eggs fixed in modified Flemming followed by nitric acid solution, 10 μ, iron-hæmatoxylin, Gage's acid fuchsin except Fig. 35, and reproduced to the scale shown below.

FIG. 26. From a single section showing both pronuclei. The second polocyte is on the next section.

FIG. 27. Reconstructed from two consecutive sections, showing both pronuclei and second polocyte.

FIG. 28, 29. Two consecutive sections. Fig. 28 contains the entire sperm pronucleus; Fig. 29, the entire egg pronucleus, except that some fragments of karomeres on the next section were not transposed. A thin layer of egg cytoplasm separates the two pronuclei.

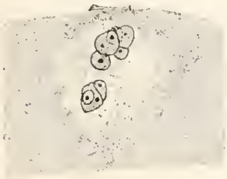
FIG. 30, 31. Two consecutive sections. Fig. 31 shows the sperm and 30, the egg pronucleus. The two pronuclei are separated by a very thin layer of cytoplasm.

FIG. 32. A fertilization stage with a supernumerary sperm pronucleus. Reconstructed from three consecutive sections. The first section contains the second polocyte, in which the chromosome vesicles have apparently disintegrated, and the disintegrating sperm pronucleus. The egg pronucleus is on the second section and the active sperm pronucleus is on the third.

FIGS. 33, 34. Two consecutive sections. Fig. 33 shows the sperm pronucleus and three vesicles of the egg pronucleus. In Fig. 34 the chromosomes of the first polocyte are arranged in monaster metaphase and are apparently disintegrating. The second polocyte has vesicles in which the chromatin is in the form of disintegrating chromomeres. A "Zwischenkörper," *Z*, connects the cytoplasm of the ovum with that of the second polocyte, as in Fig. 27.

FIG. 35. From a single section, showing the karyomeres nearly all fused to form definite pronuclei. The chromosomes of the first polocyte have undergone monocentric division resulting in an indefinite number of chromatin masses. The centrosome of the monaster is distinctly visible. Ohlmacher, 6 μ, iron-hæmatoxylin.

FIGS. 36, 37. Two of three consecutive sections. Fig. 36 shows the sperm pronucleus and 37, the egg pronucleus. The intervening section shows no trace of either pronucleus, but it does show that the hyaline region surrounding the two pronuclei is continuous and practically free from yolk granules as is shown in Fig. 33.



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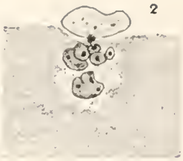

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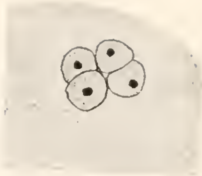

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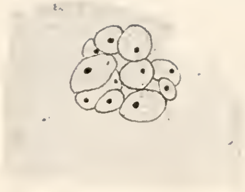

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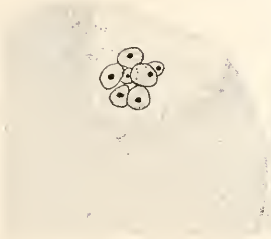

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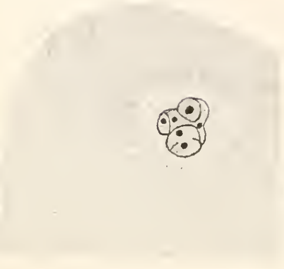

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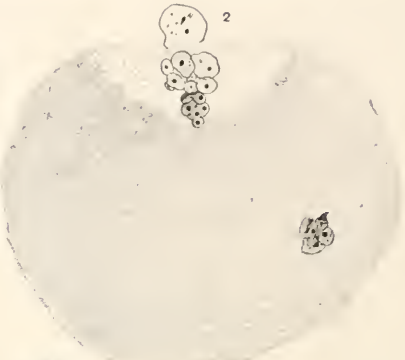

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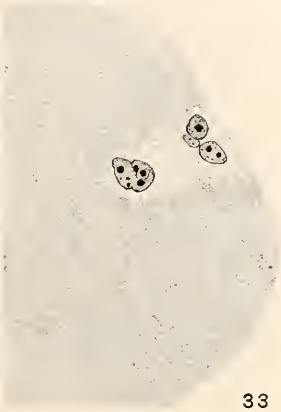

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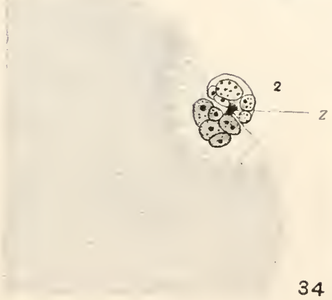

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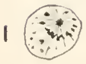

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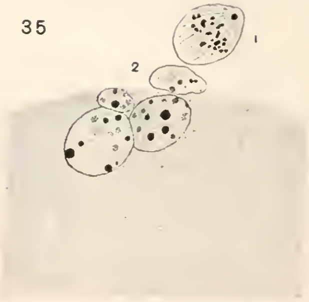

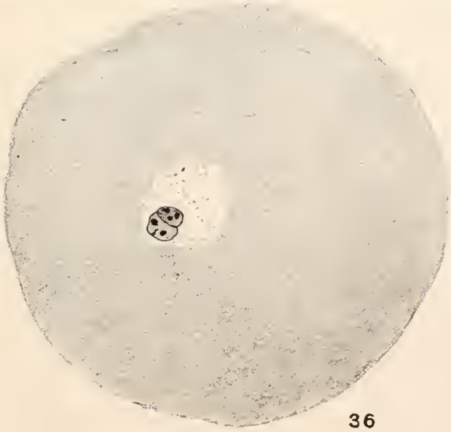

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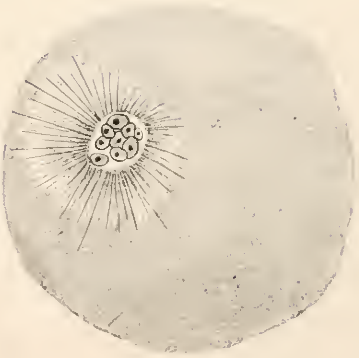

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Plate 5.

Figs. 38–43 were reproduced to the scale of 0.03 mm.; Figs. 47–51, 0.02 mm. as shown in the plate.

Figs. 38, 40. Two consecutive sections showing reduction in number of karyomeres of the pronuclei. Both sperm and egg pronuclei are represented. The polar periphery of the egg is represented by a line. Modified Flemming, 6 μ, iron-hæmatoxylin.

Fig. 39. From a single section showing sperm and egg pronuclei with two unresolved karyomeres. The polar periphery of the egg is represented by a line. Modified Flemming, 6 μ, iron-hæmatoxylin.

Fig. 41. From an oblique section through the definitive sperm and egg pronuclei; complete except that a part of the upper pronucleus is on the next section. The pronuclei are closely appressed but are not fused. Modified Burkhardt 48° C., 10 μ, iron-hæmatoxylin, Gage's acid fuchsin.

Figs. 42, 43. Two consecutive sections of the first cleavage nucleus. Only a few astral rays show in the first section. However, I supplied the amphiaster to assist in explaining both figures. Numerous chromomeres are retained in the second polocyte, and the astral rays are breaking down the nuclear membrane. Technique as in Fig. 41.

Fig. 44. A sperm pronucleus from the sperm receptacle, much enlarged. Modified Burkhardt 50° C., 6 μ, iron-hæmatoxylin.

Fig. 45. A nearly ripe ovarian ovum. Al, Ancel's layer. Projected with "Zeichenokkular."

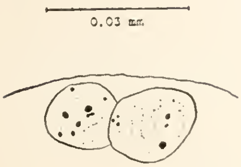
Fig. 46. A very recently freed ovum lying in an acinus and still retaining more than half of its hyaline membrane, *HM*, and its germinal vesicle. Projected with "Zeichenokkular."

Fig. 47. Reconstructed from two consecutive sections of a primary oöcyte which was deposited in the sperm receptacle during copulation. Modified Flemming, 8 μ, iron-hæmatoxylin.

Figs. 48, 50. Early equatorial plates of the first maturation in virgin eggs. Each figure was drawn from a single section and comprises 16 and 20 chromosomes each. These eggs were fixed while being oviposited. Modified Flemming, 6 μ, iron-hæmatoxylin.

Fig. 49. First maturation spindle in an egg from the same egg-mass as Figs. 48 and 50, but earlier. The entire figure is on one section. The number of chromosomes cannot be determined.

Fig. 51. Very late anaphase of the first maturation division. The bulging first polocyte is indicated by a line. Modified Flemming 65° C., 6 μ, iron-hæmatoxylin.



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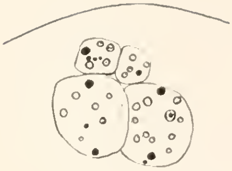

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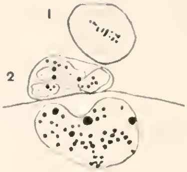

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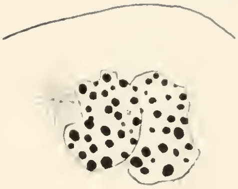

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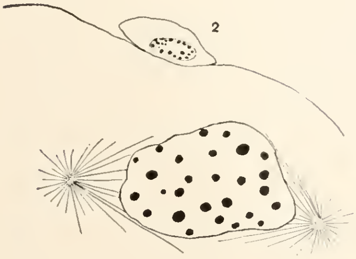

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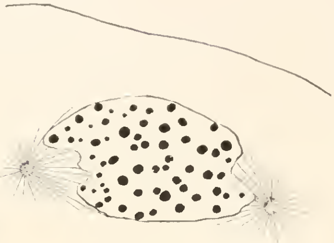

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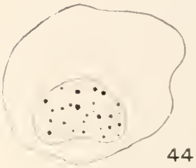

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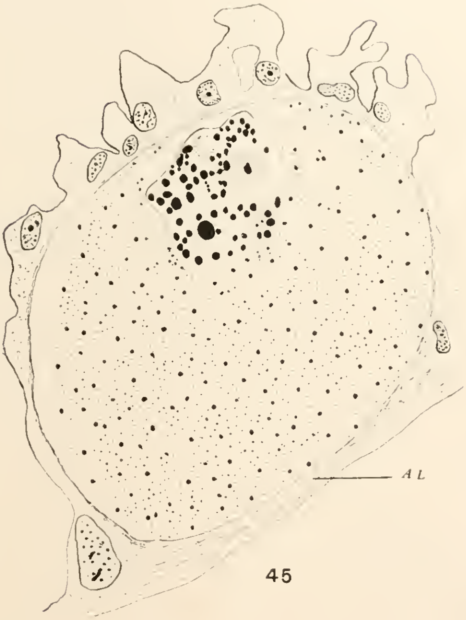

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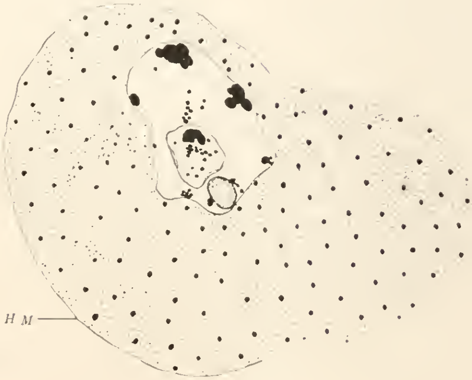

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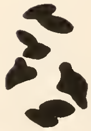

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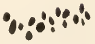

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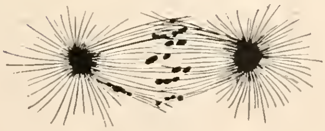

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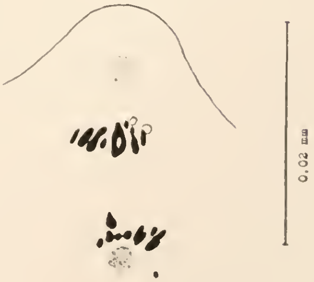


51

EDWARD DRANE CRABB.

Plate 6.

Each figure was drawn to the scale 0.02 mm. as shown.

Figs. 52, 58. First polocytes arranged according to their approximate ages. Each was drawn from a single section having all the chromosomes in it. 52–54, modified Flemming; 55, 56, 58, modified Burkhardt; 57, modified Flemming 60° C. All were cut 6 μ and stained with iron-hæmatoxylin.

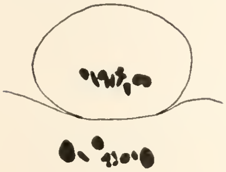
Fig. 59. A more detailed reproduction of the first polocyte shown in Fig. 35.

Fig. 60. An unusual anaphase of the second maturation division. From the section adjoining Fig. 53.

Figs. 61–63. Anaphase of the second maturation division showing progressive changes in the centrosome of the egg nucleus correlated with the movements of the daughter chromosomes. All chromosomes are from a single section except in Fig. 62 the four lagging ones of the second polar nucleus were transposed from the next section, as were those represented as circles in 63. All eggs are from the same mass and the nucleus is complete in each figure. Modified Burkhardt 50° C., 10 μ, iron-hæmatoxylin, Gage's acid fuchsin.

Figs. 64–66. Primary spermatocytes. Fig. 64, modified Burkhardt, 6 μ; Fig. 65, Perenyi, 8 μ; Fig. 66, modified Flemming, 8 μ, iron-hæmatoxylin.

Figs. 67, 68. Secondary spermatocytes. Perenyi, 8 μ, iron-hæmatoxylin.



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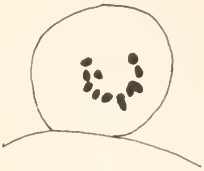

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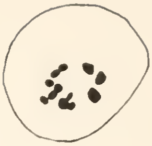

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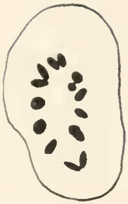

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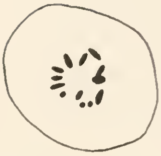

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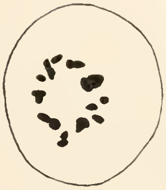

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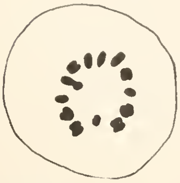

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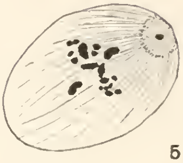

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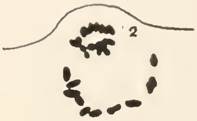

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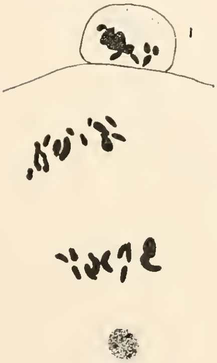

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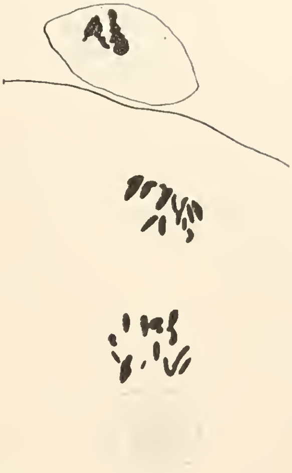

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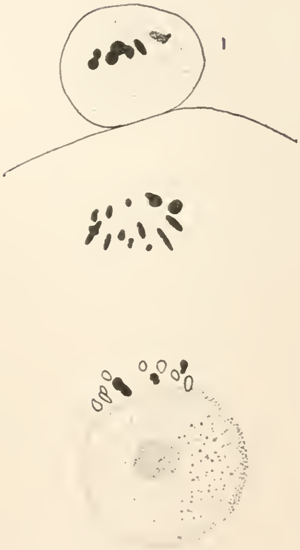

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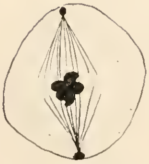

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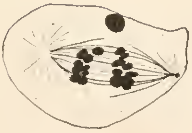

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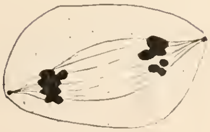

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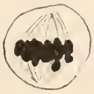

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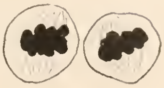

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EDWARD DRANE CRABB.

MITOSIS AND CELL DIVISION IN *EUGLENA SPIROGYRA* EHRENBERG.[1]

H. L. RATCLIFFE.

I. Introduction.

Cell division of the free-living flagellates has been studied with special reference to (1) the presence of a centriole or division center, (2) chromosome formation and division, and (3) the origin of the motor organelles following division.

Berliner (1909), Hartmann and Chagas (1910), Bělař (1916) and others have described centrioles in the mitotic process of several of the euglenoid flagellates. Tschenzoff (1916), Hall (1923), Baker (1926) and others have studied this process in others of the same group, but have found nothing which appears to be a centriole.

Chromosome formation in the prophase of mitosis has been found to vary in different members of this group of organisms. Tschenzoff (1916) found masses of chromatin forming about the periphery of the nucleus in the prophase which eventually formed chromosomes in *Euglena viridis*. In *Menoidium incurvum,* Hall (1923) found that the chromatin was organized in "thread-like structures" which shortened and thickened to form distinct chromosomes at this stage of division. In *Euglena agilis,* Baker (1926) described the chromatin granules, which, in the vegetative stage, lie on "nodes of a linin network," as fusing during the prophase to form the chromosomes.

Chromosome division in the metaphase of mitosis has been described differently by various authors. Two types have been reported—transverse fission by Hartmann and Chagas (1910), Bělař (1916) and others, and longitudinal fission by Tschenzoff (1916), Hall (1923), Baker (1926) and others.

The origin of flagella following division has also been found to vary in different forms. Hartmann and Chagas (1910) and Hall

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[1] From the Department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University. This work was begun in the Protozoölogy Laboratory, at Emory University, Atlanta, Georgia.

(1923) have described division of the blepharoplast, and Hall obtained evidence that the old flagellum split to form two new ones during cell division in *Menoidium incurvum.* The motor organelles of *Euglena agilis* have been found to disintegrate and blepharoplasts originate anew from the endosome, from which grow the flagella of the daughter organisms (Baker, 1926). In *Heteronema acus,* a biflagellated form, the original flagella persist and new flagella are produced during division, one for each daughter cell (Rhodes and Brown, MS.).

The mitotic process of *Euglena spirogyra* lends itself readily to the further study of these problems because of its large nucleus and easily studied cytoplasmic structures.

II. Material and Methods.

The euglenas were grown in finger bowls of 200 cc. capacity on a modification of Doflein's flagellate culture medium (see "Lehrbuch der Protozoenkunde, Vierte Auflage," p. 371, F. Doflein) made up in tap-water. It was found that, following exposure to several hours sunlight, division occurred in abundance, beginning about two hours after sunset, and approximately all forms dividing on any given day began fission within an hour after this time. The process was completed within 3–4 hours, so that by fixing material every half hour one obtained a well-connected picture of division.

Fleming's and Schaudinn's fluids were used with equal success. Material was killed *en masse* after centrifuging about one half a minute, and was allowed to stand in the fixative for about 30 minutes. Shorter fixation was not satisfactory. After this it was thoroughly washed in distilled water and run through the alcohol series to 85 per cent. alcohol in which it was allowed to stand three or four days to remove the chlorophyll. From the alcohol the material was fixed on slides with egg albumen and stained with aqueous iron alum hæmatoxylin, counterstaining with eosin or orange G. Other stains were used but these did not give the differentiation necessary for studying mitotic figures. The entire process of mitosis was also observed in the living material with oil immersion lens. Continued exposure to the light of the microscope caused nuclear division to cease, but after nuclear division was complete cell cleavage would continue while exposed to this light but would be greatly retarded. The lights in the laboratory, however, seemed to have no effect.

Acknowledgment.

Many thanks are due Dr. R. C. Rhodes under whose direction this work was started, and to Dr. R. W. Hegner under whose direction it was completed.

III. Mitosis.

1. *The vegetative nucleus* (Fig. 1) is of the vesicular type, described for other euglenoids by Tschenzoff (1916), Hall (1923), Baker (1926), and others, and lies mid-way of the longitudinal axis of the cell. It is elipsoidal in shape and is surrounded by a nuclear membrane (Fig. 1) which persists throughout mitosis, and which is usually drawn out toward the blepharoplast during vegetative life. The chromatin is in the form of homogeneous granules arranged in paired rows or strands that lie at random about the endosome which is centrally placed. This body is elongate and varies in appearance in different nuclei. It is enlarged about a vacuole which may occupy any position in its substance (Fig. 1, *a, b, c*), and which contains a large granule of the same staining capacity as other parts of this structure. A similar granule of practically the same size lies among the chromatin threads in some part of the nucleus which, during the prophase of mitosis, forms the blepharoplasts of the daughter organisms. At this stage it has been called the intranuclear body (Fig. 1). The significance of the former granule is not known nor can it be traced through division. It appears, however, as a constant feature of the endosome.

2. *Prophase.*—With the initiation of division the intranuclear body moves toward the anterior end of the nucleus, divides (Fig. 1, *a, b, c*), and the halves come to lie on the anterior border of the nuclear membrane. The nucleus moves forward (Fig. 2, 3, 4) into contact with the base of the reservoir. The flagellum shortens until it is drawn into the exterior opening of the reservoir (Fig. 4). The mass at the bifurcation disappears and the blepharoplasts move apart so that the axial filaments form an inverted V

(Figs. 2, 3, 4). The intranuclear bodies bud off masses which pass through the nuclear membrane to the base of the reservoir where they become the blepharoplasts of the daughter organism. New axial filaments (Fig. 4) grow out from these to unite with the original ones (Fig. 5), and the flagellum splits longitudinally, thus forming the new flagella. The connections between the nuclei and the blepharoplast persist long after division is completed (Fig. 14)—in fact they apparently persist throughout the vegetative stage.

The chromatin threads shorten and thicken (Figs. 2–5) until the chromosomes become homogeneous bodies no longer granular in appearance (Fig. 6). The chromosome pairs are never arranged in a typical equatorial plate as is characteristic of mitosis of higher forms. The vacuole within the endosome disappears (Fig. 2–4). Then the endosome elongates at right angles to the long axis of the body (Fig. 4–6).

3. *Metaphase.*—The chromosome pairs, which, for the most part, lie in planes parallel to the endosome (Fig. 7), begin separation and many forms show the typical V-shapes with the ends of the V more or less closely joined, characteristic of this stage of mitosis in some higher forms. The endosome continues elongation and appears to be fragmented into two or more pieces which lie somewhat twisted about each other (Figs. 6, 7).

4. *Anaphase.*—The chromosome pairs separate and move to opposite poles of the nucleus (Fig. 8). As this takes place the individual chromosomes undergo a longitudinal fission and resolve into the rows of paired granules in which condition they pass the remainder of the life cycle. This anaphasic division of the chromosomes accounts for the paired condition seen in other stages of the life cycle. The nuclear membrane constricts closely following the movement of the chromosomes to the poles of the nucleus (Fig. 8).

5. *Telophase.*—Nuclear division is completed with the final constriction of the endosome and the nuclear membrane (Figs. 9, 10). The endosome rounds up in the center of the mass of chromosomes which change very little appearance during this stage. Following the constriction of the nuclear membrane there is an invagination of the base of the reservoir which continues

anteriorly until it finally divides the reservoir into halves (Figs. 9, 10, 11). When this is completed the anterior end of the organism widens so that the mouths of the two reservoirs become separated (Fig. 11); then division of the body begins at the anterior end proceeding posteriorly in a line between two cuticular striations (Figs. 12, 13). The body and body contents are thus divided into two approximately equal parts (Fig. 14).

6. *Reorganization.*—Following cell division the organism undergoes very little change for some time. The nucleus remains attached to the belpharoplasts (Fig. 14) as it slowly moves posteriorly. The chromatin becomes less condensed slowly assuming the appearance of the vegetative stage. The endosome buds off a portion of its substance to form the intranuclear body (Fig. 14) which remains unchanged until the succeeding mitotic division, and the central vacuole reappears, small at first (Fig. 14) gradually becoming larger until the usual size is reached.

III. Discussion.

1. *Division Center.*—This study of the mitosis of *E. spirogyra* sheds little light upon the problem of the division center or centriole of the free-living flagellates. The mitotic process takes places within the nuclear membrane and if any structure, present at this time, is comparable to the division center of other forms, it is the endosome. The structure may also be comparable to the spindle and centrosomes of higher types but nothing has been found that would convince one that it contains a division center.

2. Chromosome formation and division in *E. spirogyra* is quite similar to that of metazoan types. The chromosomes form, in the prophase, from the paired strands of chromomeres into paired chromosomes of the metaphase, which separate and undergo a longitudinal fission in the following anaphase. In the daughter nuclei, during the telophase, they resolve into the vegetative state in which they are arranged as paired rows of granules or chromomeres. Thus for this form at least we have an example of mitosis in the protozoa that bears out "one of the most fundamental conceptions of cytology and genetics, namely, that the spireme threads are linear aggregates of much smaller self-perpetuating bodies, aligned in single series and definite order"

(Wilson, 1925). One cannot say just how far the nuclear mechanism of this organism might bear out the principles of genetics that have been proven in metazoan types, but the observations seem to indicate that the chromosomes are composed of "self-perpetuating bodies" aligned in a single series since the chromosome elements undergo a definite linear fission each time the cycle is completed.

3. Origin of the motor organelles of *E. spirogyra* is quite similar to that reported for *E. agilis* Baker (1926). The mass from which the kinetic elements arise is budded off from the endosome during the period of reorganization following division, while Baker found that in *E. agilis* this mass was given off in the prophase, and, instead of the rhizoplast persisting as in *E. spirogyra,* the mass leaves the nuclear membrane to form the "kineto-nucleus" (Baker, 1926). Concerning this phenomenon in *E. agilis* Baker concludes, "The endosome in *E. agilis* is the ultimate source of all the kinetic elements of the cell. . . ." The same may be said for *E. spirogyra* although the kinetic element is budded off at a different time in the life cycle.

Splitting of flagella during division has been described by Steuer (1904) for *Eutreptia,* and Hall (1923) reported the same for *Menoidium incurvum.* One may easily see that the axial fibers of *E. spirogyra* move apart during the prophase of division thus splitting the old flagellum. As pointed out in the introduction, Baker (1926) states that these organelles are lost during division and originate anew for each daughter cell following division of *E. agilis.*

IV. Summary.

Nuclear division in *Euglena spirogyra* takes place within the nuclear membrane, and no centriole appears during the process. The nucleus moves forward into contact with the base of the reservoir. The chromatin in the vegetative nucleus is in the form of paired strands of chromomeres. These shorten and thicken in the prophase and lose their granular appearance, forming the chromosome pairs of the metaphase. The chromosomes are never arranged in a true equatorial plate, but the members of each pair move apart more or less individually in the anaphase and each chromosome undergoes a longitudinal fission as it resolves again into the granular state. The nuclear membrane constricts in the mid line following the movement of the chromosomes to the poles of the nucleus. The endosome lies in the center of the chromosome mass throughout the process. It becomes homogeneous early in the prophase; then elongates at right angles to the long axis of the body and constricts in halves preceeding the complete constriction of the nuclear membrane. It resumes its vegetative appearance in the reorganization period.

The kinetic elements of the flagellum are derived from the endosome and lie in the nucleus during the vegetative life as the intranuclear body. Prior to division this divides; then moves to the nuclear membrane and, as the nucleus comes into contact with the base of the reservoir, the halves give rise to the blepharoplasts. Two new axial filaments grow out, one from each new blepharoplast, and unite with the original axial filaments. The axial filaments then become widely separated, splitting the original flagellum as they move apart. The two flagella for the daughter organisms are thus formed and grow out to their normal length following division. The rhizoplast connecting the nucleus to the blepharoplasts persists late into the vegetative stage and possibly throughout the whole of this period.

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EXPLANATION OF PLATES.

Plate I.

All figures drawn with camera lucida. Fig. 1 drawn at a magnification of 1,100 diameters. All other figures drawn at a magnification of 825 diameters.

Fig. 1. Vegetative form of *Euglena spirogyra*. Chromatin at this time in the form of paired rows of chromomeres. All other structures to which reference is made are labeled.

Fig. 1, *a, b, c*. Early prophase stages showing forward movement and division of intranuclear body.

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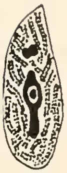

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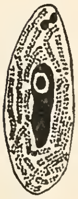

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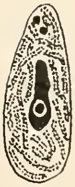

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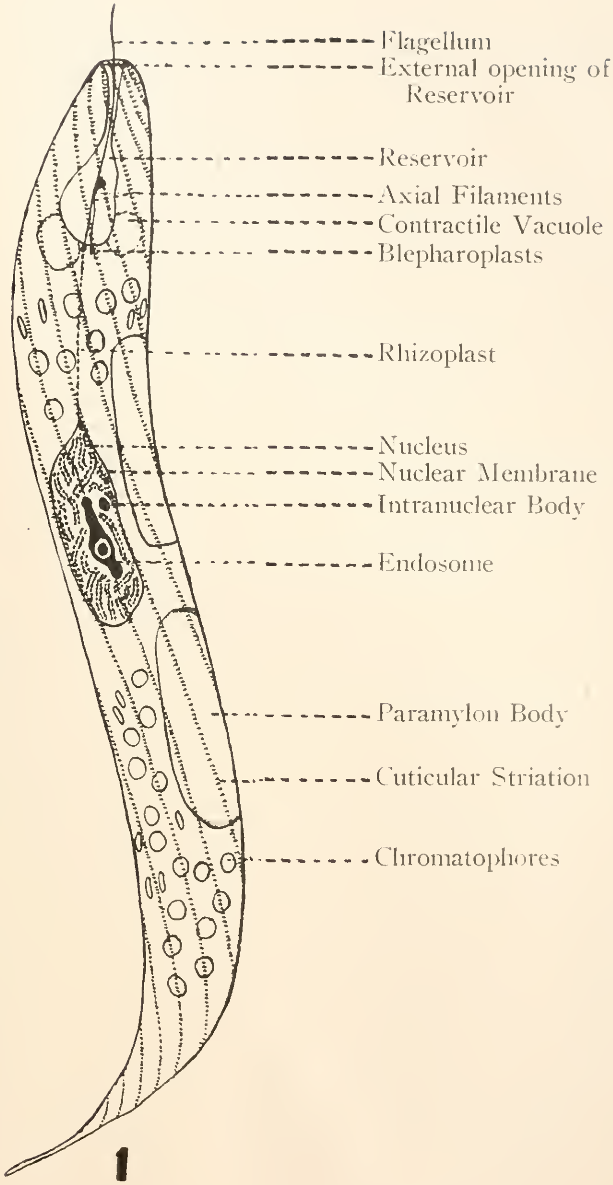

Flagellum
External opening of Reservoir
Reservoir
Axial Filaments
Contractile Vacuole
Blepharoplasts
Rhizoplast
Nucleus
Nuclear Membrane
Intranuclear Body
Endosome
Paramylon Body
Cuticular Striation
Chromatophores

1

H. L. RATCLIFFE.

PLATE 2.

FIG. 2–3. Nucleus moves forward and changes from elipsoidal to ovoidal. Chromatin threads shorten and thicken. Intranuclear body has divided and lies on the nuclear membrane. Flagellum is retracted, the mass at the bifurcation becomes smaller as the blepharoplasts move apart.

FIG. 4. Nucleus in contact with the base of the reservoir. The new blepharoplasts are formed from the divided intranuclear body, definite rhizoplasts connect them to the nucleus, and new axial fibers grow out from them. The old blepharoplasts are wide apart. The flagellum begins splitting and the mass at the bifurcation disappears. The endosome becomes condensed in appearance and the vacuole disappears. The chromatin threads continue shortening and condensation.

FIG. 5. Chromosomes are quite condensed and their granular nature is not so pronounced. The endosome begins elongation at right angles to the long axis of cell and is composed of two parts. The new axial fibers have united with the old ones and the flagellum has completely divided.

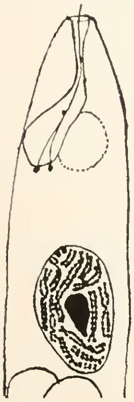
FIG. 6. Chromosomes are uniform in appearance, lying in pairs about the endosome, which continues elongation and is composed of three parts.

FIG. 7. The metaphase. Chromosome pairs separate. Endosome continues elongation and is composed of two parts.

FIG. 8. The anaphase. Constriction of nuclear membrane following movement of chromosomes to poles of nucleus. Chromosomes undergo longitudinal fission and become granular again.

FIG. 9. The telophase. The endosome divides. Anterior border of nuclear membrane drawn out to a point as the nucleus moves posteriorly. Invagination of base of reservoir begins.

FIG. 10. Daughter nuclei separated. Reservoir division completed. Anterior end of organism widens.



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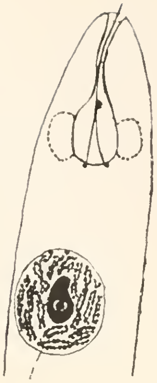

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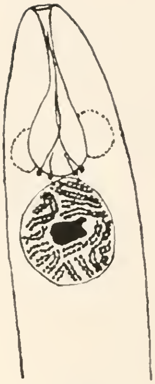

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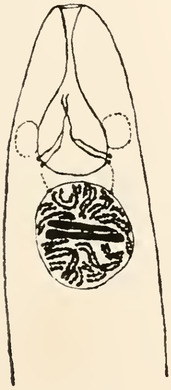

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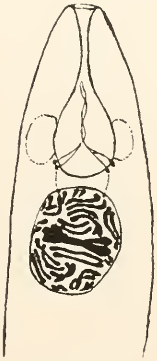

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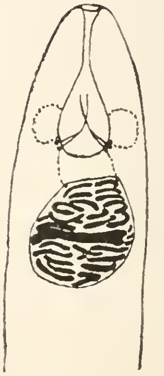

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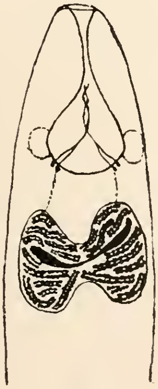

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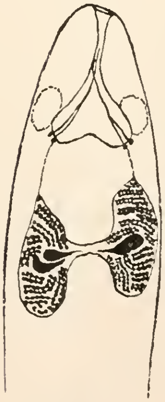

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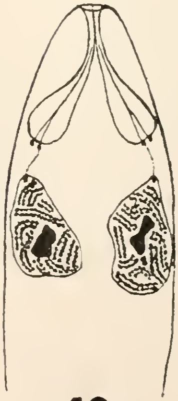

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H. L. RATCLIFFE.

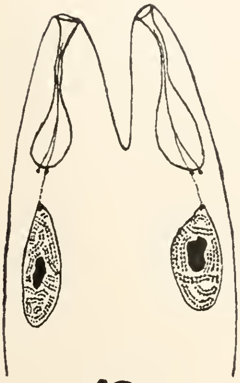
PLATE 3.

FIG. 11. Widening of anterior end continues.

FIG. 12. Cell division begins.

FIG. 13. Cell division at a late stage.

FIG. 14. Reorganization. Cell division complete. Chromatin resumes the vegetative appearance of paired strands of chromomeres. Endosome buds off intranuclear body and the vacuole in the endosome reappears.



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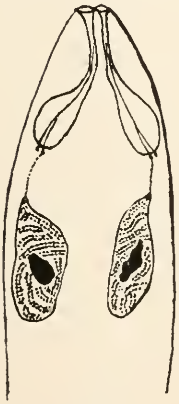

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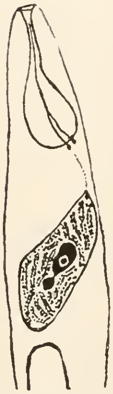

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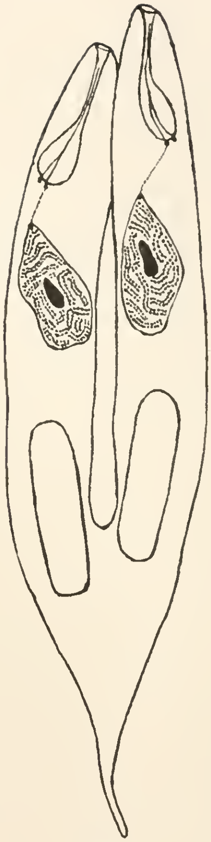

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H. L. RATCLIFFE

THE NERVOUS SYSTEM OF PELAGIC NEMERTEANS.

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During the past ten years a considerable number of species of bathypelagic nemerteans have been studied anatomically by Brinkmann ('17) and others and much information has been secured, showing the deviations of the nervous system from the general plan characteristic for the littoral forms. Five new genera, collected by the "Albatross" in various parts of the Pacific ocean, which have recently been fully studied by the writer (Coe, '26), proved of particular interest in this respect. One of these species, *Neuronemertes aurantiaca,* possesses a chain of ganglia along the dorsal nerve, a condition almost unique in invertebrates.

In the modifications of the sense organs also the pelagic nemerteans (Pelagica) differ widely from any of the littoral and bottom-living species (Reptantia).

It is to be recalled that bathypelagic nemerteans have been found only in the open oceans, where they swim with sluggish movements or float idly, but always far beneath the surface. Certain of the species are known to be restricted to particular water layers, usually at depths of from 500 to 1,500 meters, and presumably all the species are limited in their vertical range by the conditions of temperature and salinity, and to some extent, perhaps, by the pressure, to which they have become specifically adapted. Their geographical range, on the other hand, may be very wide, since water layers of essentially similar physical and chemical properties extend through vast areas of the oceans and almost from pole to pole. It is in conformity with these environmental conditions that the bathypelagic organisms have evolved.

Up to the present time 47 species of these aberrant worms have been described, but it is quite possible that some of them may be synonyms. More than half of them have been carefully studied with respect to their anatomical peculiarities, including those of the nervous system. Stiasny-Wijnhoff ('23) has also described a form which was taken from the bottom at a depth of 833 meters, which is in some respects intermediate between the Pelagica and the Reptantia.

Brain.—In Fig. 1 a general plan of the nervous system is shown. The brain of the pelagic forms is essentially similar to that of the littoral species, but as a rule the dorsal ganglia are considerably smaller than the ventral. The two ganglia of each side are closely fused together. The fibrous cores and the three types of ganglion cells are also similar, but neurochord cells have not been found in any species.

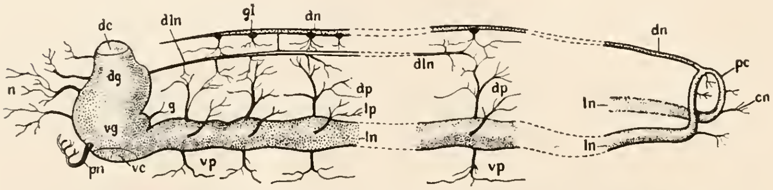

FIG. 1. Diagram of nervous system of *Neuronemertes aurantiaca* Coe, showing relation of brain and lateral nerves (*ln*) to peripheral nervous system; *dg*, dorsal, and *vg*, ventral ganglia of brain; *dc* and *vc*, the corresponding commissures; *n*, cephalic nerves; *g*, gastric nerve; *pn*, proboscidial nerve; *dn*, dorsal nerve, with metameric ganglia (*gl*); *dln*, dorsolateral nerve; *dp*, *lp*, *vp*, dorsal, lateral and ventral peripheral nerves, respectively; *pc*, posterior commissure; *cn*, caudal nerves.

One of the most striking peculiarities of the pelagic forms is the great amount of gelatinous tissue, or parenchyma, which surrounds all the internal organs, giving the body a high degree of permeability and a low specific gravity, enabling the worms to float freely in the water at great depths with a minimum of muscular exertion. This parenchyma surrounds the brain and usually separates it widely from the thin musculature of the head.

The probosis passes through the ring made by the ganglia and their dorsal and ventral commissures, while the stomach lies beneath the ventral commissure in most species.

Three longitudinal nerve stems extend the entire length of the body; the pair of lateral nerves, situated in the body parenchyma beneath the intestinal diverticula, and the median dorsal nerve, which lies directly beneath the basement layer of the epidermis. The former are direct continuations of the ventral brain lobes, while the dorsal nerve has no direct connection with the brain

(Fig. 1). The fibrous cores of these main nerves are connected at the posterior end of the body by the broad posterior commissure, which passes on the dorsal side of the rectum. They are also connected indirectly metamerically by the union of minute branches from both dorsal and dorsal peripheral nerves in the intermuscular plexus (Figs. 1, 2, 4).

Dorsolateral nerves.—In the anterior part of the body of many of the species studied a pair of nerves originating from the dorsal brain lobes, pass backward along the internal border of the body musculature near the dorsolateral edges of the probosis sheath. These dorsolateral nerves (Figs. 1, 2) are connected by means of delicate fibres with both the dorsal nerves and the dorsal periphereal nerves of the lateral nerve cords. Efferent branches supply the body musculature on the dorsolateral aspects of the body, as well as the proboscis sheath. These nerves are evidently homologous with the dorsolateral nerves of other Platyhelminths. They do not occur in all the pelagic species, and where present are limited to the anterior end of the body (Fig. 1). In other species, and toward the middle of the body in all species, their place is taken by fibres from the dorsal peripheral nerves.

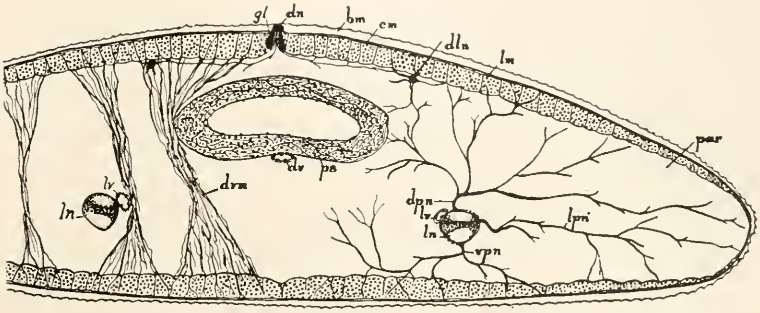

FIG. 2. Portion of transverse section of body of *Neuronemertes aurantiaca* Coe, showing on one side the relations of the peripheral nerves to the body musculature. The section passes between adjacent diverticula of the intestine and shows on the left hand side a portion of the dorsoventral musculature (*dvn*); *dn*, dorsal nerve, with bilobed ganglion (*gl*); *dpn*, *lpn*, and *vpn*, dorsal, lateral and ventral peripheral nerves; *ps*, proboscis sheath; *dv* and *lv*, dorsal and lateral blood vessels; *bm*, basement layer underlying the surface epithelium; *par*, parenchyma; *cm* and *lm*, the thin circular and longitudinal musculatures of the body walls. Other letters as in Fig. 1.

Dorsal nerve.—This nerve appears to represent a local concentration of fibers from the dorsal peripheral nerves, for it is not connected directly with the brain, so far as known, in any of the pelagic forms. It extends in the median line and just external to the circular muscular layer, joining the commissure of the lateral nerves at the posterior end of the body. When followed forward toward the brain it becomes gradually smaller and finally terminates in the cephalic parenchyma or in the delicate intermuscular plexus a short distance posterior to the dorsal brain commissure (Fig. 1).

Fibers from the dorsal nerve supply the dorsal musculature and integument of the body. Other fibers enter the nervous plexus lying between the two muscular layers, while still others are connected with the dorsolateral and dorsal peripheral nerves.

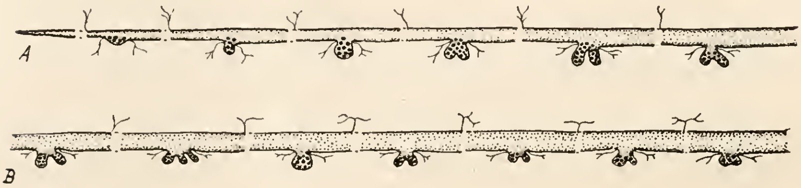

Fig. 3. *A*, Diagram of anterior end of dorsal nerve with its accompanying ganglia, showing variations in shape of the latter and the minute fibrils which pass dorsally into the integument and ventrally into the intermuscular plexus. *B*, Similar diagram from near middle of body.

Ganglia of dorsal nerves.—In most species only a few scattered ganglion cells are to be found along the course of the dorsal nerve, but in *Neuronemertes aurantiaca* this nerve is very large and is provided with a series of nervous structures, apparently of the nature of ganglia, although such organs have not hitherto been found in any species of nemertean. In my monograph on the Pelagic Nemerteans (Coe, '26) they were referred to as "problematical organs" although their probable nature as ganglia was emphasized (p. 130).

The number of these organs is upward of a hundred, corresponding presumably to a primitive metamerism.

Anteriorly the ganglia are somewhat smaller than the diameter of the fibrous core of the dorsal nerve, but farther back in the body they are several times as large as the nerve and are often divided into two or more lobes (Figs. 3, 4). The nerve itself lies external to the circular muscular layer, while the ganglia are internal to these muscles and interposed between the bundles of longitudinal muscles. The broad connection between ganglion and nerve therefore requires the penetration of the thin circular musculature. This is accomplished by the mere separation of adjacent circular fibers so as to leave a narrow slit through which the connection passes.

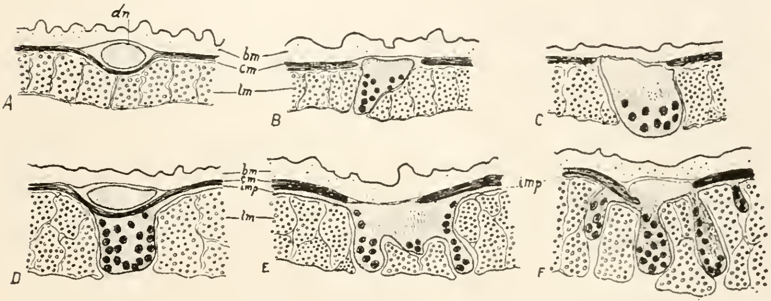

Fig. 4. Transverse sections of six of the ganglia of the dorsal nerve of *Neuronemertes aurantiaca* Coa, showing variations in shape and their relations to the nerve core. *A*, Dorsal nerve between two adjacent ganglia. *B*, Small ganglion closely fused with dorsal nerve. *C*, Large ganglion with ventral group of nerve cells. *D*, Surface view of ganglion, its connection with dorsal nerve being in an adjacent section. *E*, Large ganglion with three lobes of nerve cells and distinct intermuscular plexus. *F*, Large ganglion with four lobes of nerve cells; *imp*, intermuscular plexus; other lettering as in Fig. 2.

The cells composing the ganglia are similar in size and appearance to those which accompany the lateral nerve cords, and the cell boundaries are likewise rarely to be distinguished (Fig. 4). It has been difficult to determine the precise nature of these organs. That their component cells are the source of fibers which innervate the integument and musculatures seems highly probable. And it is also presumable that they supplement the nerve cells found in all species accompanying the lateral nerve cords, but there is no evidence that these latter cells are less abundant in this species than in other pelagic forms. A suggestion that may have some degree of plausibility is that they are associated with the great development of the dorsoventral musculature, particularly in the region where the spermaries are situated in the male, just behind the head. In *Neuronemertes* the walls of the spermaries are not provided with the thick spiral musculature found in several other pelagic forms and which serves to supplement the weak muscles of the body walls in the forcible discharge of the spermatozoa. A similar result is accomplished however by the contraction of the highly developed dorsoventral muscles which closely invest the spermaries in that genus. It therefore seems reasonable to suspect that these supplementary nervous tissues may function in the control of this dorsoventral musculature throughout the body.

There still remains the possibility of the ganglia being correlated with sensory stimuli rather than with motor responses, but their structure is unlike that of any known receptor. It seems necessary, therefore, to return to the view that the ganglia of the dorsal nerve supplement the nerve cells of the lateral nerve cords just as these supplement those of the brain.

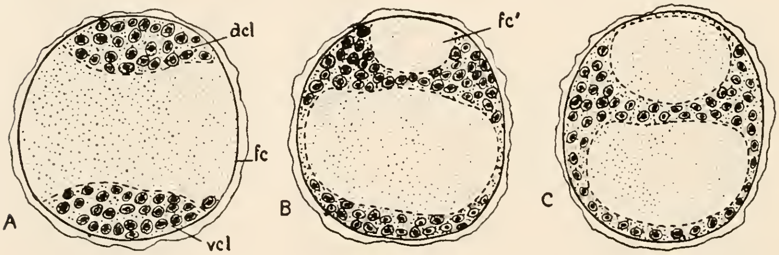

FIG. 5. Transverse section of lateral nerve cord in three different genera, showing the relations of the fibrous cores to the accompanying nerve cells. *A, Plionemertes plana* Coe, with single fibrous core (*fc*) and symmetrical dorsal and ventral cellular layers (*dcl* and *vcl*) ; *B, Proarmaueria pellucida* Coe, with small dorsal core (*fc'*) ; *C, Planonemertes lobata* Coe, with large dorsal core.

Lateral nerves.—These are direct continuations of the fibrous cores of the brain lobes and are accompanied by nerve cells throughout their entire length. At the posterior end of the body the cords of the two sides form a broad commissure on the dorsal side of the rectum (Fig. 1). In most pelagic species the fibrous core arising from the dorsal brain lobe is separated by a layer of ganglion cells, at least in the anterior part of the body, from that arising from the ventral lobe of the brain (Fig. 5).

The dorsal core is much smaller than the ventral and its separation from the latter tends to become less distinct in the posterior half of the body. Toward the posterior end of the body this core becomes gradually smaller, only the fibers of the ventral core being involved in the posterior nerve-commissure.

In a few genera there is but a single fibrous core, and this arises mainly from the ventral ganglion. It presumably also contains fibers from the dorsal ganglion, although they are usually not visibly separated from the others (Figs. 5, 8).

The position which the lateral nerves occupy in the body varies greatly according to the degree of development of lateral margins. In those forms in which the body is but little flattened, the nerve-cords lie near the lateral margins of the body, but in the broad, flattened forms they are far removed from the lateral margins, being in some cases situated more than half-way toward the median line (Fig. 6). In nearly all species the nerve-cords bend more or less sharply laterally immediately after their origin from the brain. In most forms they lie in the parenchyma near the ventral surface of the body, but in those cases where large ventral branches from the intestinal diverticulum force their way beneath the nerve-cord, the latter is moved dorsally until it may lie about midway between dorsal and ventral surfaces (Fig. 6).

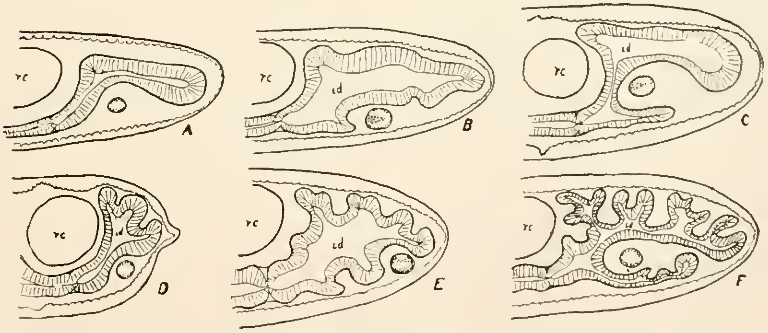

Fig. 6. Portions of transverse sections of body of six different genera, showing the position of the lateral nerve cord with respect to the body walls and intestinal diverticula. *A, Pelagonemertes joubini* Coe; *B, Planonemertes lobata* Coe; *C, Neuronemertes aurantiaca* Coe; *D, Cuneonemertes gracilis* Coe; *E, Nectonemertes pelagica* Cravens and Heath; *F, Planktonemertes agassizii* Woodworth.

Nerve cord muscle.—In the genera belonging to the families *Armaueriidæ* and *Pelagonemertidæ* and in *Pendonemertes* of the *Protopelagonemertidæ* a narrow band of muscular fibers splits off from the proboscis sheath on each side of the body immediately back of the brain and extends backward throughout the length of the body in close connection with the lateral nerve cord. In some forms it lies close against the outer connective sheath on the median face of the nerve cord, while in other cases it penetrates the sheath and comes to lie in close connection with the nervous tissues. This muscle is usually very thin, consisting of only one or two layers of muscular fibers (Figs. 7, 8*A*), but in a few species it is so large that it equals fully half the diameter of the nerve cord itself (Fig. 8*B*).

In *Pelagonemertes joubini* the muscle leaves the proboscis sheath as several separate strands (Fig. 7) and these may at first take separate positions on the face of the nerve cord, but further back in the body they fuse into a single flattened bundle.

The function of the nerve cord muscle is evidently to hold the nerve cord in position with reference to the organs which the latter innervates.

Peripheral nerves.—As a rule three peripheral nerves leave each lateral nerve cord in each of the spaces between adjacent intestinal diverticula. One of these three nerves, dorsal peripheral, leaves the dorsal side of the cord (Figs. 1, 2, 7, 8) to supply the dorsal musculature and integument and in some cases the proboscis sheath (Figs. 2, 7). This nerve also sends branches into the intermuscular plexus and indirectly connects with the dorsal and dorsolateral nerves. Small branches also supply many of the dorsoventral muscles.

The lateral peripheral is a slender nerve with few branches. These supply the lateral margin of the body and appear also to enter the intermuscular plexus (Figs. 2, 7).

The ventral peripheral originates from the ventral core of the cord and divides into two branches which supply the ventral portions of the body. Branches from this nerve also enter the ventral intermuscular plexus (Figs. 1, 2, 7, 8).

Intermuscular plexus.—In some of the sections of *Neuronemertes aurantiaca* distinct nerves can be seen leading from the dorsal nerve inward through the circular muscular layer and thence laterally between the two muscular layers of the body-walls. These nerves appear to constitute part of a great plexus of nerve-fibers which extends between the circular and longitudinal musculatures throughout the length of the body, both

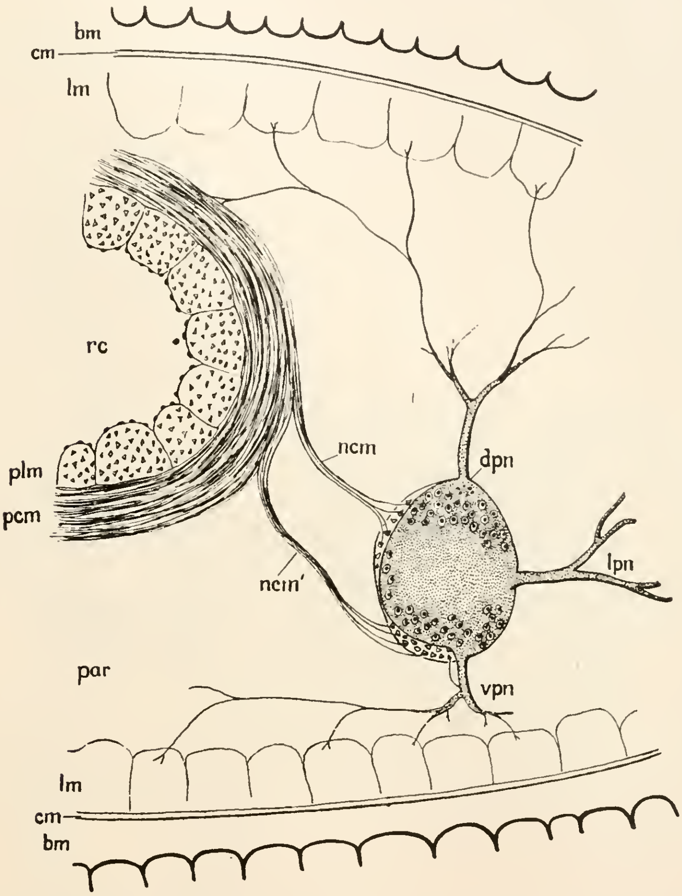

Fig. 7. Diagram of portion of transverse section of body of *Pelagonemertes joubini* Coe, immediately posterior to the brain, showing origin of the nerve cord muscles (*ncm, ncm'*) from the outer spiral musculature of the proboscis sheath, and the three main branches, dorsal (*dpn*), lateral (*lpn*), and ventral (*vpn*) which leave the nerve cord in each interdiverticular space; *rc,* rhynchocoel; *plm* and *pcm,* longitudinal and circular-spiral muscular layers of proboscis sheath; other lettering as in Fig. 2.

dorsally and ventrally (Fig. 4). The plexus is in frequent communication with the peripheral nerves from the lateral cords, as well as with the dorsal and dorsolateral nerves.

Tentacular nerves.—The adult males of *Nectonemertes* and both sexes of *Balænanemertes* are each provided with a pair of tentacles immediately back of the head. These appendages are lateral outgrowth of the body walls and are supplied with large nerves homologous with the lateral peripheral nerves in other parts of the body (Fig. 9).

Proboscis-sheath nerves.—In many pelagic species the proboscis sheath is supplied with nerves which enter it at the ring where the proboscis is attached, but in other forms the organ also receives one or more pairs of fine branches from the dorsal peripheral nerves (Figs. 2, 7).

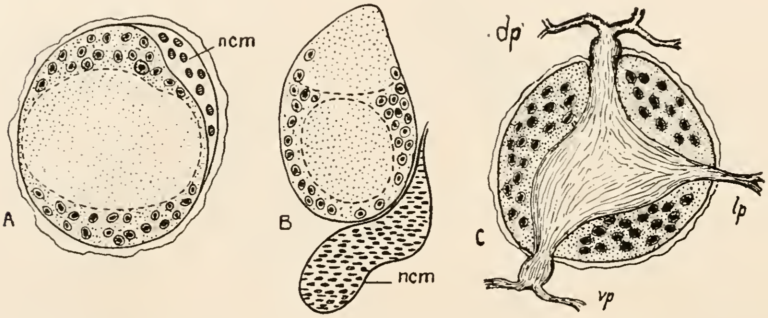

FIG. 8. *A,* Transverse section of lateral nerve cord and accompanying muscle (*ncm*) in *Cuneonemertes gracilis* Coe. *B,* Same in *Balænanemertes chuni* Bürger. *C,* Section of lateral nerve cord in *Planktonemertes agassizii* Woodworth, showing the origin of dorsal lateral and ventral peripheral nerves.

Proboscidial nerves.—The proboscis is large and highly specialized in most of the pelagic nemerteans, with a powerful musculature and a thick epithelial lining of secretary and sensory cells in addition to the minute sickel-shaped weapon with which it is provided. Consequently it requires an abundant supply of nerves. In some forms these are provided by means of a pair of large nerve stems arising from the points where the ventral commissure joins the ventral brain lobes (Fig. 1). This pair of large nerves divides into the definite number of proboscidial nerves either at the attachment of the proboscis or in the organ immediately posterior to its insertion. But in other forms, where the proboscis is inserted close in front of the brain, the proboscidial nerves either arise in the complete number from the ventral brain lobes and the commissure adjacent or branch immediately after their origin.

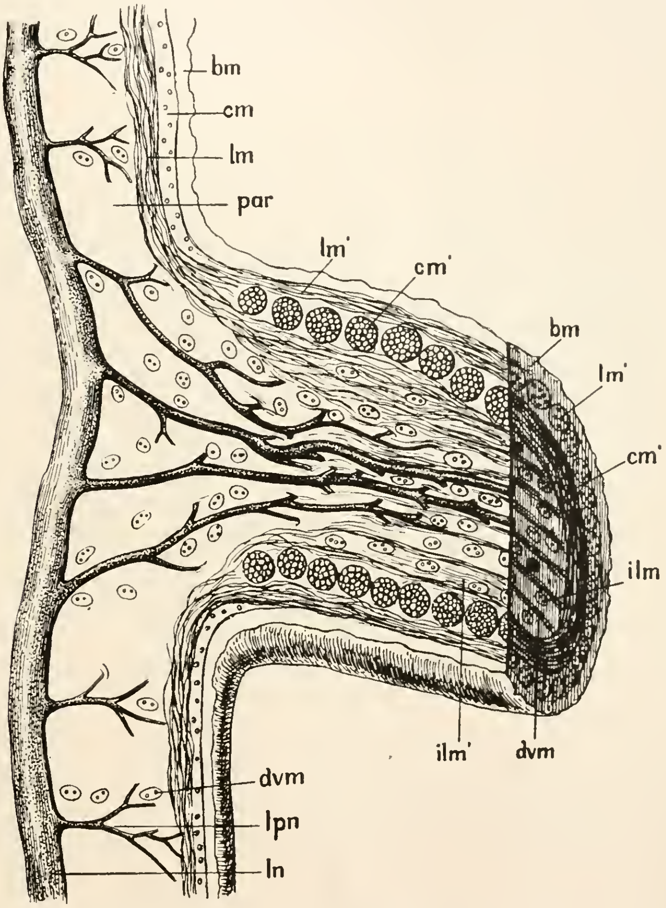

Fig. 9. Diagram of portion of horizontal section of tentacle and adjacent body wall of *Nectonemertes mirabilis* Verrill, showing the four large tentacular nerves originating from the lateral nerve cord (*ln*). These are homologous with the lateral peripheral nerves (*lpn*) of the rest of the body; *bm*, basement layer underlying the surface epithelium; *lm*, longitudinal and *cm*, circular muscular layer; *ilm*, internal longitudinal muscles; *dvm*, dorsoventral muscles; *par*, *parenchyma*.

The nerves take up a position in the midst of the longitudinal muscular layer along the whole circumference of the proboscis (Fig. 10). This position varies somewhat in the different forms, but is generally about two thirds the distance from the inner to the outer border of the longitudinal muscular layer. Here the nerves branch out to form a thick plexus of connecting fibers and send symmetrical branches radially both towards the periphery and towards the glandular epithelium lining the central lumen (Fig. 10).

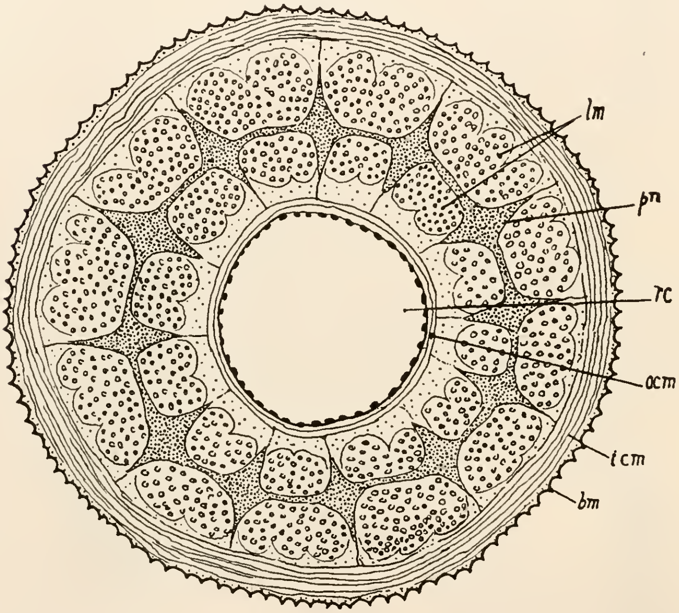

Fig. 10. Transverse section of everted proboscis of *Cuneonemertes gracilis* Coe, with the 12 proboscidial nerves (*pn*) connected by a plexus in the midst of the longitudinal muscular layer (*lm*); *icm* and *ocm*, inner and outer circular musculatures; *bm*, basement layer underlying the inner, grandular epithelium; *rc*, extension of the rhynchocoel; lined with endothelium.

About midway between each two proboscidial nerves in certain species the plexus is condensed into a secondary nerve, commonly about half the diameter of one of the main nerves. There is thus an equal number of primary and secondary nerves in such species. In some forms it is difficult to distinguish the two sets of nerves, so that one specimen may appear to have double the number found in other specimens where the proboscis is in a different state of contraction. Furthermore in some forms the number of nerves in the anterior end of the proboscis may be double that found farther back toward the stylet region, due to the gradual disappearance of each alternate, or secondary, nerve in the plexus. And, finally, the number may vary to some extent in different individuals, as in *Nectonemertes mirabilis* where the number ranges from eighteen to twenty-four.

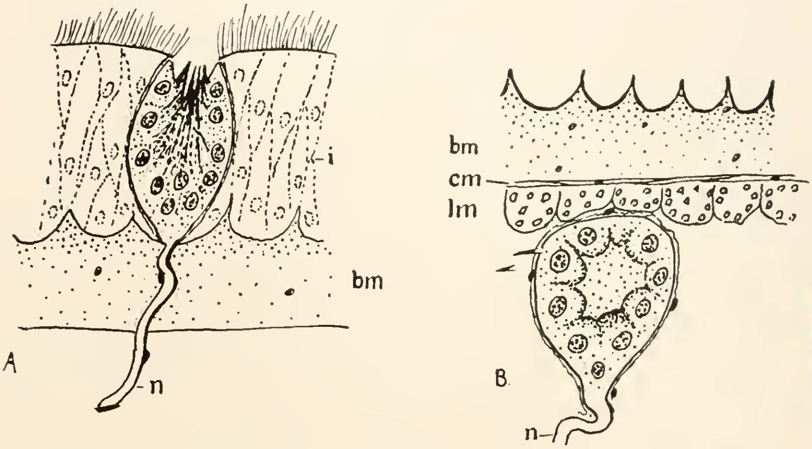

FIG. 11. Diagrams of the two types of sense organs found in *Cuneonemertes gracilis* Coe. *A*, integumentary sense organ; *B*, subcutaneous sense organ; *bm*, basement layer of surface epithelium (*i*); *cm* and *lm*, circular and longitudinal musculatures of cephalic wall; *n*, nerve leading to dorsal brain lobe.

The central radial branches from the nervous plexus pass between the bundles of longitudinal muscles and form a second, delicate intermuscular plexus beneath the inner circular muscles. From this plexus fine nerve-fibers supply the epithelium of the papillæ as well as the inner circular muscles.

The peripheral radial branches arising from each of the primary nerves form a third, still more delicate, plexus between the longitudinal muscles and the outer circular muscular layer. This supplies in a similar manner the more superficial layers of the proboscis, namely, the outer circular muscles and the endothelium.

Of the three nervous plexuses (middle, inner, and outer) which are thus formed the two latter are of such great delicacy that they can be observed only under favorable conditions of preservation, and it is by no means certain that they are present in all species.

Although the radial nerves leave the middle plexus only at intervals, the three plexuses appear to form more or less continuous networks throughout the length of the anterior chamber. In some forms even the middle plexus is incompletely developed, the primary proboscidial nerves being connected with each other only at intervals.

The plexuses are interrupted in the middle chamber of the proboscis by the interlacing of the muscular layers, and the primary nerves gradually lose their identity in a multitude of smaller branches, some of which innervate the stylet musculature and gland-cells, while others continue backward into the wall of the posterior chamber. Hence in the posterior chamber the primary nerves are not usually distinguishable as separate units and there is but a single plexus lying between the longitudinal muscles and the glandular epithelium lining the lumen.

The smallest number of proboscidial nerves found in any pelagic species is 7, and the largest is 50, the most frequent numbers being from 15 to 25. In general, the species in which the individuals attain the largest size have a greater number than those in which the animals are but a few millimeters in length. Odd and even numbers are about evenly distributed.

Cephalic nerves.—The muscles, integument and sense organs of the head are supplied with a considerable number of fine nerves which arise from the anterior surfaces of both dorsal and ventral brain lobes (Fig. 1). But since the cephalic musculature is extremely weak and the sense organs rudimentary, the cephalic nerves are very much smaller than in most other nemerteans.

Gastric nerves.—A pair of small nerves arise from the posterior end of the ventral brain lobes to supply the anterior end of the digestive tract (Fig. 1). These are homologous with the esophageal nerves of the littoral forms which have the esophagus well developed, but where that part of the digestive system is rudimentary, as in the pelagic species, the nerves supply the stomach. Supplementary gastric nerves, originating from the ventral commissure, have also been observed in one or more species.

Pyloric nerves.—In some species, at least, a pair of minute nerves, originating from the lateral nerve cords, enters the anterior end of the pylorus.

Caudal nerves.—The posterior extremity of the body is supplied with nerves in two different ways. In *Proarmaueria* the posterior end of each of the lateral nerves divides into two branches, the larger of which enters the posterior commissure, while the smaller supplies the extremity of the body. In *Neuronemertes* and some other forms the caudal extremity is supplied with small nerves from the commissure itself (Fig. 1).

Sense-organs.—Even the most primitive of the littoral nemerteans are provided with special sense-organs as differentiated portions of the integument, and in the higher groups ocelli, cerebral sense-organs, and frontal sense-organs are of frequent occurrence. In addition, otocysts are found in a few species. But in the pelagic forms no such organs have been found in any of the species studied. Possibly vestiges of one of these types of sense-organs were discovered by Bürger ('09) in *Balænanemertes*. These are considered by him to be rudimentary ocelli, and similar organs were shortly afterward found by Brinkmann in *Pelagonemertes rollestoni*. Further studies have shown them to be widely distributed among the *Armaueriidæ* and *Pelagonemertidæ*. They are of two distinct types, as follows:

Subcutaneous sense-organs.—In these forms a small cluster of sense-organs, whatever their nature may be, is situated beneath the basement layer of the anterior margin of the head, frequently on either side of the rhynchodeal opening. Each of the end-organs consists of a pear-shaped group of cells, connected with a branch of one of the cephalic nerves (Fig. 11*B*). Some of them lie in contact with the inner border of the basement-layer, while others may be situated somewhat deeper in the cephalic tissues, and in or even beneath the muscular layers. Since they differ widely from typical nemertean ocelli in structure, it seems quite possible that these may be special sense-organs peculiar to the pelagic forms rather than that they represent degenerate ocelli.

Integumentary sense-organs.—A second type of sense-organ occurs in the integument of the head and less frequently in other parts of the body in certain genera. These consist of compact groups of sensory cells imbedded in the integument. The cells are provided with slender distal processes and each group is supplied with a nerve leading to the brain or to the lateral nerve, according to its location (Fig. 11*A*). It is not improbable that such integumentary sense-organs occur in the majority of the pelagic species, but that the loss of the integument during capture has prevented their discovery.

SUMMARY.

Correlated with their usually gelatinous consistency, weak musculature and other adaptations for life far beneath the surface of the oceans, the nervous system of the bathypelagic nemerteans (Pelagica) differs considerably from that found in the littoral and bottom-living relatives (Reptantia). Comparisons are made between the Pelagica and Reptantia with respect to each of the principal parts of the nervous system. The special sense-organs of the Reptantia are either lacking entirely or merely vestigial in the bathypelagic forms. With particular reference to a recently described species, *Neuronemertes aurantiaca:*

1. The dorsal nerve is not connected directly with the brain.
2. The dorsal nerve is provided with metameric ganglia, not previously known for any nemertean.
3. A pair of dorsolateral nerves connects both with the dorsal nerve and with dorsal peripheral branches of the lateral nerve cords.
4. A delicate intermuscular plexus lies between the two body musculatures.

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THE LIFE HISTORY OF *PLANARIA VELATA*.

W. A. CASTLE.

Previous investigators working with a common flatworm, *Planaria velata,* have observed that the ordinary type of life history of the species is an entirely asexual one (Child, '13). Following the emergence of the young animals from cysts is a period of growth which is followed by processes of histological degeneration and loss of internal structure as soon as the animal has attained a certain size. This is followed by fragmentation of the worm and the encystment of the fragments. The external aspects of this process have been mentioned by Stringer (Stringer, '09) and more fully described by Child (Child, '13). Observations on the histological details of the process of degeneration have been made by Alexander (Alexander, '26). Our observations confirm those of Alexander in regard to the details of this process.

From the encysted fragments new worms are reconstituted and emerge from the cysts after a period of from two weeks to several months. The time necessary for complete reconstitution is dependent upon the conditions to which the cysts are subjected. Asexual reproduction may continue in this way for an apparently indefinite number of generations (Child, '14) and until comparatively recently has been the only observed method of reproduction for the species.

Under certain conditions in nature *Planaria velata* has been observed to undergo a life history and course of development entirely different from the asexual one mentioned above. Accompanying growth there has been the differentiation of sexual structures. Under the most favorable conditions sexual development has been completed and the sexually mature worms have copulated and laid eggs. Although none of these eggs laid in the laboratory hatched there is no reason to doubt that they would under favorable conditions develop after the manner of those of other planarians; however sufficient material has not yet been available to make possible a study of the embryonic stages.

Following sexual maturity or with the advent of unfavorable conditions before sexual differentiation is complete, it has been observed that the worms undergo a histological degeneration similar to that which precedes the fragmentation of an asexual stock. This may at first involve only the structures associated with the reproductive system. In this event the worms return to the asexual state and a period of growth may ensue before the ultimate degeneration that leads to fragmentation and encystment. Under conditions that are more decidedly adverse to sexuality a degeneration of the other internal structures may accompany that of the sex organs, and fragmentation and encystment will follow without any intermediate period of asexuality and growth.

It has been found that the life cycle of *Planaria velata* may be altered and controlled by subjection to the proper experimental conditions. This is true not only of the asexual cycle but of the sexual cycle as well. In nature the conditions bringing about encystment are a period of good nutrition and growth accompanied by an increase in the temperature of the water. Child observed that by partial starvation the onset of fragmentation and encystment might be warded off indefinitely. Worms were thus maintained at approximately the same size and in approximately the same physiological condition for more than two years (Child, '14).

Keeping the worms at a lower temperature has been found to prolong the period of growth that precedes encystment but not to inhibit encystment for an indefinite period of time. At room temperatures (18–20° C.) worms will cease feeding and proceed towards encystment when they have attained a length of twelve or fourteen millimeters, whereas worms kept in an ice box at 8–10° will attain a length of eighteen or twenty millimeters before the onset of fragmentation. Eventual encystment however is the result always to be expected when the temperature remains constant. Conversely it is found that at temperatures higher than normal worms can be induced to encyst at a much smaller size. The approach of encystment can be detected in advance by the cessation of activity, loss of pigmentation, and the disintegration of the pharynx. The fate of worms in such a condition can be altered by manipulation of the temperature to which they are subjected. A rise in temperature will hasten the onset of fragmentation; lowering of the temperature will retard it. In some cases worms with degenerated pharynges have been prevented from encystment by a change in temperature from that of the room to 8°. These worms have regenerated pharynges, acquired more pigment, and become perfectly normal asexual worms at the lower temperature.

Sexuality has never been observed in specimens of *Planaria velata* collected from temporary pools or ditches. It seems quite probable that conditions in that kind of habitat are never favorable to the development of sexual structures.

In a spring at Valparaiso, Indiana, *Planaria velata* has been found to occur at all months of the year. During October, November, and December the worms are increasing very rapidly in numbers and in size. These worms presumably had their origin in cysts formed by the fragmentation of worms as the water became warmer the preceding spring. In the latter part of November and through December all of the worms of a length of eight to ten millimeters or over show a very distinct genital pore. Microscopical examination shows testes and ovaries are present. The height of sexual development is reached by the latter part of December. The largest worms collected then show not only a complete development of ovaries, testes, and atrial organs, but in some of them the vasa deferentia are well developed and packed with spermatozoa.

From mid-winter on there is a decrease in the number of sexual individuals as well as in the number of worms to be found. By the middle of March there is no trace of sex organs in any of the worms collected. Through the months of April and May the worms decrease greatly in abundance, the larger ones undergoing fragmentation and encystment and the smaller ones remaining, so that during the summer months only a few small or medium sized asexual worms are found.

The most evident variable factor that might be effective in influencing the sexual cycle in this location at Valparaiso is seen at once to be temperature. During the months of September and October when the small worms are emerging from cysts there is a gradual fall in the temperature of the water. The maximum reached during the summer is about 12–13° C. By the end of October this has fallen to 8–9°. The minimum is reached by the end of December, and at that time completely differentiated sexual worms have been collected from beneath the ice where the water is no warmer than 5°. During late January and early February there is a pronounced decrease in the number of sexual worms before there is any appreciable rise in the temperature of the water. During the spring there is a rise in water temperature accompanied by the total disappearance of sexual structures. The majority of the worms fragment and encyst during these months of rising temperature; some however persist through the summer as asexual worms.

Experiments have been carried out to determine the extent to which sexuality is influenced by changes in temperature. First it is noted that when sexual worms are brought from their natural habitat and placed in the laboratory at a temperature of 18–20° there is a quite rapid disappearance of sexual structures. This occurs irrespective of food conditions. By the end of two weeks ovaries, testes, and atrial organs have completely vanished. This is followed at once by the fragmentation and encystment of all the larger worms. Medium sized and small worms become apparently normal asexuals, but if fed will grow and undergo the usual precystic changes as soon as they have attained a sufficient size.

The history of the majority of sexual stocks placed at a temperature of 8–10° is not different from this, although if the animals are not fed very few of them will encyst unless they are exceptionally large. The disappearance of sexual structures is much retarded—testes and a genital pore may remain evident for from four to six weeks after the date of collection. With the possible exception of one lot of worms further sexual differentiation has never been observed after the time of collection. In this single case worms which were exceptionally large and well differentiated sexually were isolated at 8° at the time of collection. Three eggs were laid by these worms within twelve days of the time of isolation. Subsequently to this the worms lost all sexual structures and eventually fragmented and encysted in the usual manner. It is seen from these experiments that low temperature retards the disappearance of sexual structures once they are developed. We may now consider the possibility of experimentally developing sexual worms from an asexual stock by the manipulation of the temperature to which the worms are subjected.

That there might be no question of a genetic difference of strains producing sexual and asexual worms, stocks having their origin from cysts have been taken as the starting point of these experiments. Such worms when fed at room temperature have invariably undergone a period of asexual growth and eventually encysted without any suggestion of approaching sexuality. At temperatures ranging from 4 to 12° the result is the same if the worms are placed at that temperature soon after emergence from cysts and the temperature is maintained fairly constant throughout the period of growth. As might be expected growth at low temperatures is much less rapid and the worms attain a much greater size before encystment. These experiments show that sexuality is not to be expected at either high or low temperatures as long as the temperature is constant throughout the life of the animals.

Asexual worms raised to a length of ten or twelve millimeters at room temperature and then subjected to a temperature of 8–10° have repeatedly been found to show indications of approaching sexuality. In such worms ovaries and testes in advanced stages of gametogenesis have been found within two weeks of the time of the change in temperature. Atrial organs have been practically completely developed. In all of these cases the worms have been so small that complete sexual development was not expected. In none of the experiments resulting in the development of sexual worms have the animals been fed during the time while the sex organs were differentiating. It has been observed that with the resumption of feeding there is at first a slight further development of the sex organs that is followed by their degeneration and the return of the worms to the asexual condition. A change from room temperatures to temperatures of from 3 to 8° has not been found effective in bringing about sexual development. Such experimental stocks have continued slow asexual growth when fed but have shown no development of sexual structures. It is apparent from the results of these experiments that temperature is an important factor in the control of sexuality in Planaria velata. From observations of the conditions under which sexuality is known to occur in nature it is probable that the rate of growth is also important. Experiments along these lines are being continued.

Histological studies have been made upon the differentiation of the sexual structures as well as upon the anatomy of the reproductive system of sexually mature worms. Other studies have been made upon the degeneration of sexual structures and the changes that accompany and precede either fragmentation or a return to the asexual condition. The structure of encysted fragments and the process of reconstitution within the cyst have been made the subject of further histological investigation. The encysted fragments consist of a layer of epithelial cells forming a sac which encloses a mass consisting largely of globules of fats, proteins, etc., but containing a few scattered nuclei. Some degree of protoplasmic continuity evidently exists throughout this mass, for in the process of reconstitution the pharynx arises from the middle of the mass at a considerable distance from any part of the wall. The region of the developing pharynx first becomes distinguishable as an area containing rapidly increasing numbers of nuclei. It has not been determined whether the axis of the new worm is a result of the gradient present in the old worm which has persisted through the processes of fragmentation and encystment or the result of a new gradient that has arisen because of conditions to which the cyst is subjected.

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THE DEVELOPMENT OF THE SOLDIER CASTE IN THE TERMITE GENUS *TERMOPSIS*.

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As is well known to the student of the subject, two theories have been proposed to account for the origin of termite castes. According to the first view the young are all alike at the time of hatching and subsequently are modified by extrinsic factors. The second view supports the claim that even before hatching two castes at least, the reproductive and worker or the soldier, can be distinguished by differences in certain of the internal organs. In an effort to determine which of these two theories is the correct one it appeared that the most favorable line of approach to the solution of the problem is through the study of recently established colonies of *Termopsis* where all of the young belong to the soldier caste. With such material the junior author confined his investigations to *T. angusticollis,* while the senior author directed his attention to *T. nevadensis*. As a result the number of molts has been established, the characteristics of each instar have been determined and a comparison of the two species at the close of our independent investigations indicates that the history of both species is, in all essential particulars, the same.

The number of young appearing in a colony varies according to the nature of its surroundings. Primary royal pairs, occupying burrows in very damp situations, have been found without either eggs or young at the end of eleven months. On the other hand in what appear to be optimum conditions there may be as many as twenty-one individuals of various instars, including one or two fully developed soldiers as well as several eggs in various stages of development. This being the case, a section of the pine belt along the southern shore of Monterey Bay was kept under observation, since the conditions throughout were not only fairly uniform but highly favorable as well. Records kept of census counts in this district during a period of twenty years may therefore be considered to indicate with a fair degree of accuracy the rate of increase from year to year. In 19 nests, examined in July and August (the months when colonies normally are established), the number of individuals, not including unhatched embryos, varied from 2 to 17. These probably belonged to communities not more than one year of age. In 16 other associations the number ranged from 31 to 47. Since these were collected during the late summer, and no intermediate numbers connected them, it is reasonable to conclude that these larger colonies were two years old. In 11 cases the membership ranged from 97 to 121; these, it is believed, are three years of age. Finally there were 14 colonies numbering 327 as a minimum and 497 as a maximum—probably a four-year development.

Whether these figures are strictly correct or not, it is certain that in the early years of colony development the rate of increase in the number of offspring of *Termopsis* is comparatively slow. Even in old colonies, where the royal pair is surrounded by a thousand or more descendants, the egg tubes never become so numerous that they reduce the queen to an inert reproductive sac as in the case of certain tropical species. Hence it is safe to assume, on the basis of extended observations, that a community with 450 members is certainly three years of age if not four as the above figures indicate.

As previously mentioned, no individual with wing buds has been discovered in young colonies. To be more explicit none are known to appear before the members of a community total approximately 450. In the case of *T. nevadensis,* for example, there were two nymphs of this type in a colony numbering 454 individuals, 6 were in another colony of 467 members, 7 in one with 497 and 39 in one with a total of 490. Beyond this point the number of winged individuals mounts fairly rapidly until in old colonies, judging from the extent of the burrows as well as the size of the population, they may outnumber those of the soldier caste. In the case of *T. angusticolis* the same feature exists, but from data in hand the winged individuals put in an appearance at a somewhat later date when the number of inhabitants is nearer 500.

Before entering upon a detailed description of the development of the soldier caste it is of importance to note that practically all of our observations rest upon the study of small colonies which never exceeded a hundred individuals. The size of the burrows they inhabited also gave evidence of comparatively young communities. Furthermore the brains of nearly two hundred individuals, chiefly in the first instar, were carefully measured and the plotted results showed the same type of variation curve as did the head width measurements. About fifty gonad measurements were made which likewise showed the same type of variation as width of head and brain. These facts, coupled with the entire absence of any insect which showed even a trace of wing buds, renders it perfectly certain that the following observations relate solely to the soldier caste.

The newly laid eggs of *T. nevadensis* are approximately 1.4 mm. long by 0.57 mm. in greatest diameter, while those of *T. angusticollis* average 1.55 mm. in length by 0.58 mm. Diameter. This remains true even of those colonies where the royal pair is accompanied by hundreds of descendants, and doubtless are provided with a relatively generous food supply. As development advances all eggs increase in size at practically a uniform rate until at the time of hatching the eggs of *T. nevadensis* are approximately 1.5 mm. long by 0.6 mm. in diameter, and those of *T. angusticollis* are on the average 1.7 mm. in length by 0.68 mm. in diameter. In certain exceptional cases these lengths may be exceeded by as much as thirteen per cent., though this is generally accompanied by a proportional decrease in diameter.

Recently hatched individuals of both species are found to have the mid-gut filled with air. Whether this is swallowed or is secreted by the intestinal wall has not been determined, but it is reasonably certain that the resulting increase in body size enables the young insect to burst the egg membrane and escape. In a relatively short time the air disappears, and in several cases it was found that shortly after this stage the young are fed either by the royal pair or by some of the attendant older offspring.

Correlated with the fairly uniform size of the eggs of each species there is a corresponding uniformity in the dimension of the newly hatched young. This condition of affairs however usually undergoes a rapid change with the distention of the abdominal wall due to food in the digestive tract and the development of the fat body, and in the later stages of this first instar, when the insect is about to molt, the body occasionally may become even larger than the smallest individuals of the succeeding instar. Body length, therefore, is not an absolutely dependable criterion for the determination of a given instar. Considerably better is the customary method of determining instars on the basis of antennal segments. This likewise is not always a trustworthy guide, especially in the later stages; but it has been found that where these antennal joint counts are combined with the measurement of the transverse diameter of the head the difficulties of distinguishing the various stages practically disappear.

In recently hatched young of *T. nevadensis* the head width averages 0.648 mm. on the basis of 182 individuals taken from 25 colonies the minimum being 0.58 mm. and the maximum 0.67 mm. The number of antennal joints is usually thirteen though twelve may occur. In some specimens this variation may appear on opposite sides of the same individual. The long third joint appears to be unsegmented, but in specimens treated with caustic potash and stained with magenta this segment is found to be subdivided into two incipient segments of which the distal one is the smaller. The first instar in the case of *T. angusticollis* is practically a duplicate of the same stage in *T. nevadensis.* The transverse head diameter, however, is somewhat greater. In 116 individuals from twenty-four separate colonies the maximum width was 0.81 mm., the minimum 0.74 mm. with an average of 0.7 mm. The number of antennal segments is fourteen.

The distribution of the spines over the body of recently hatched individuals in young colonies of *T. nevadensis* is shown in Pl. I, Fig. 1; of *T. angusticollis* in Pl. II, Fig. 7. Even in the oldest communities, headed by numerous substitute royal insects, there are no appreciable differences where individuals of the first instar are concerned. In other words the newly hatched young of each species are alike externally whether they belong to a recently established community or one that has existed for several years. An exception to this rule may be noted in the case of young hatched in colonies headed by old primary royal pairs or by substitute royal forms. In such cases the spines show the same general arrangement as in younger colonies, but they are relatively longer, more slender and more flexible. This feature tends gradually to disappear as development progresses, yet spines of this character can be found to persist even into the penultimate instar.

Measurements of 192 individuals in the second instar, in the case of *T. nevadensis,* possessed a head width of 0.77 mm. in 13 specimens 0.86 mm. in 71 examples, 0.92 mm. in 78 individuals, 0.96 mm. in 28 others while 2 measured 1.0 mm. From among these 50 individuals were selected, treated with caustic potash, stained with magenta and compared. In every instance there were fifteen antennal segments; and the distribution of the spines over the body closely conformed to plan represented in Pl. I, Fig. 2. In the case of *T. angusticollis* the average head width amounted to 1.026 mm. with the usual range of variation. The number of antennal joints was sixteen; otherwise in its general proportions and distribution of spines the individuals of this instar closely resemble those of the same stage in *T. nevadensis* (Pl. II, Fig. 8).

Out of 117 individuals of *T. nevadensis* in the third instar the average head width amounted to 1.22 mm. The number of antennal segments was seventeen in 48 out of 50 specimens, the exceptions being due to the lack of the normal division between the third and fourth joints. The general appearance of the insect at this stage is represented in Pl. I, Fig. 3. In regard to *T. angusticollis* the average head width of individual in the third instar amounts to 1.3 mm. The number of antennal segments is almost invariably eighteen. Further details are shown in Pl. II, Fig. 9.

The fourth instar in *T. nevadensis* is characterized by an average head width of 1.65 mm. on the basis of 161 individuals. In the majority of instances the number of antennal joints was nineteen although 11 out of 50 had developed eighteen on one or both sides. For the distribution of the spines see Pl. I, Fig. 4. Comparing the same stage in *T. angusticollis,* the average head width amounted to 1.8 mm., and with few exceptions the number of antennal joints was twenty. Other external features are represented in Pl. II, Fig. 10.

The average head width of 136 specimens in the fifth instar was 2.35 mm. in the case of *T. nevadensis.* The number of antennal segments varied from nineteen to twenty-one. In specimens of *T. angusticollis,* in the same stage of development, the transverse head diameter was 2.56 mm., and the number of antennal segments was twenty-two in the greater number of instances, although twenty or twenty-one was not an uncommon count.

Fully developed soldiers in recently established colonies are subject to a wider range of variation than is the rule regarding any other instar. In some instances the head width is but slightly greater than that of individuals in the penultimate instar; and the number of antennal joints may be that of the foregoing stage or even less, occasional insects having but nineteen segments. The average head width of 26 soldiers of *T. nevadensis* was 2.64 mm., while 18 soldiers of *T. angusticollis* measured 2.96 mm. in transverse diameter. The number of antennal joints varied from twenty to twenty-three or even twenty-four.

From the foregoing data it becomes evident that the two species under consideration are structurally closely related, and in each instance their development follows essentially the same path. Yet they have never been found to interbreed in a state of nature, and everywhere each colony of either species invariably appears to maintain its integrity. The number of antennal joints in the same instar is, with rare exceptions, one more in the case of *T. angusticollis,* and its greater head width is also a distinctive feature, so much so in fact that even without the royal pairs the two species can be distinguished without much difficulty. It may be added that the arrangement of the spines over the body has not been found to be of much diagnostic value even where the various instars are involved. Each additional molt adds to the number of bristles, but their location is much the same in both species.

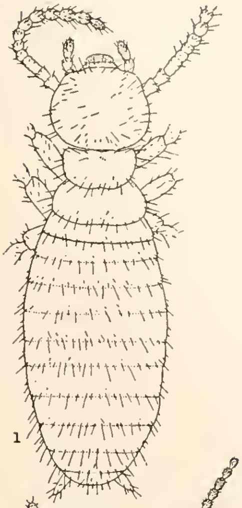
As noted previously, this study deals only with the development of the soldier caste in relatively young colonies. In older communities, where the reproductive caste has put in an appearance, the history of the soldier is somewhat more complicated. To what extent this is true can only be determined on the basis of much more extended observations, including experimental work, measurements of head, brain, gonad and perhaps other anatomical characters, a study that is well under way with a strong probability that some of the more important problems can be solved within the next few months.

PLATE I.

Explanation of Figures.

Successive stages in the development of the soldier caste in a young colony of *Termopsis nevadensis*.

FIG. 1. Newly hatched young; head width 0.65 mm.
FIG. 2. Second instar; head width 0.96 mm.
FIG. 3. Third instar; head width 1.0 mm.
FIG. 4. Fourth instar: head with 1.73 mm.
FIG. 5. Fifth instar; head width 2.18 mm.
FIG. 6. Fully developed soldier; head width 2.6 mm.



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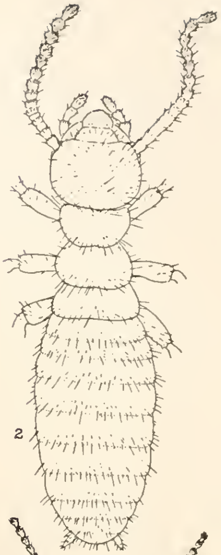

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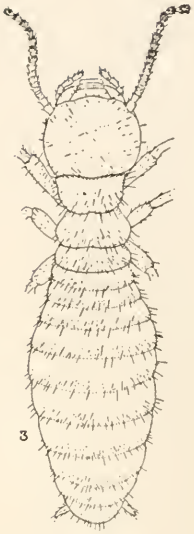

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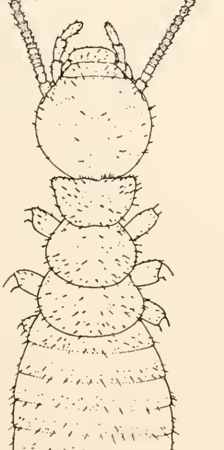

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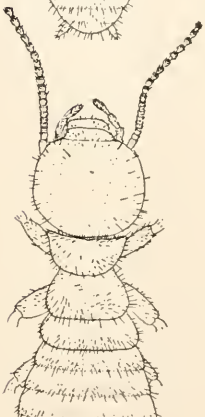

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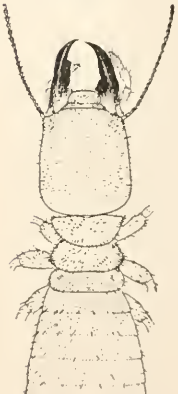

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PLATE II.

Explanation of Figures.

Successive stages in the development of the soldier caste in a young colony of *Termopsis angusticollis*.

FIG 7. Recently hatched young; head width 0.72 mm.

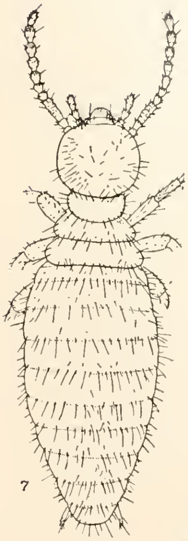
FIG. 8. Second instar; head width 1.0 mm.

FIG. 9. Third instar; head width 1.24 mm.

FIG. 10. Fourth instar; head width 1.79 mm.

FIG. 11. Fifth instar; head width 2.6 mm.

FIG. 12. Fully developed soldier; head width 2.97 mm.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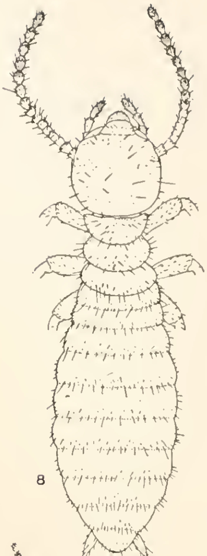
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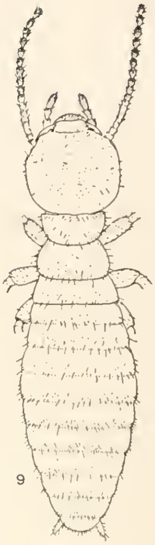
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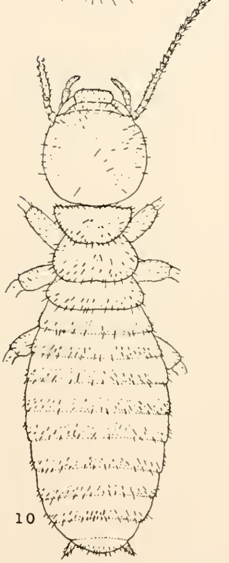
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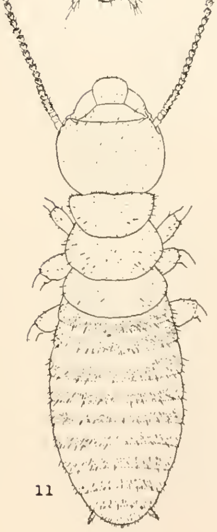
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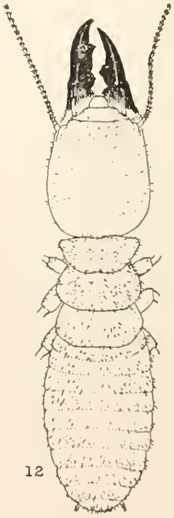
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MATURATION PHENOMENA IN ARTIFICIAL PARTHENOGENESIS OF *ASTERIAS FORBESII*.*

H. W. BEAMS.

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INTRODUCTION.[1]

Recent studies in artificial parthenogenesis of eggs that are immature when activated has resulted in several views as to the mode of maturation which may lead to normal development. Professor Tharaldsen found as a result of his previous observations of the maturation phenomena in *Asterias forbesii,* which may be artificially activated before the maturation process is completed, that this process was subject to wide variations. He proposed therefore that I make a study of the maturation phenomena in Asterias to ascertain the cytological details of the various types.

---

* Contribution from the Zoölogical Laboratory, Northwestern University, Evanston, Ill.

[1] I wish to acknowledge my indebtedness to Professor C. E. Tharaldsen for the use of his material and his kind assistance during the progress of this work. I also wish to express my appreciation of the helpful suggestions given by Professor A. E. Cole and Mr. G. B. Pickwell.

Material and Method.

The material on which these observations were made consisted of serial sections of *Asterias forbesii* eggs fixed in sublimate acetic (2.5 per cent. acetic acid), sectioned at 5 μ, and stained in Heidenhain's hæmatoxylin. The eggs were procured, artificially activated, and the cytological technique performed by Professor C. E. Tharaldsen during the summers of 1921–22–23, at the Marine Biological Laboratory at Woods Hole. The activating agents used consisted of the single butyric acid method as described by Lillie, and Loeb's double method.[2] The eggs had been induced to mature uniformly by a special technique similar to that described by Miss Brailey ('23). They were all activated at the same time and specimens were then fixed at intervals of one minute throughout the maturation from the breaking down of the germinal vesicle to the first cleavage. The slides were studied in chronological order.

Observations.

Normal Maturation.—The primary oöcyte of *Asterias forbesii* ranges in diameter from 100 μ, to 125 μ; and the nucleus of such eggs varies from one third to one half the diameter of the cell (Fig. 1). The nucleus is usually eccentrically located and the nucleolus is found in the nucleus on the side nearest the periphery of the egg. When activated during the breaking down of the germinal vesicle, the nucleus shows a highly chromatic alveolar network; and within this network is a very delicate achromatic (linin) reticulum. The nuclear wall begins to wrinkle and dissolve away at a point nearest the periphery of the egg. The dissolution of the nuclear membrane extends away from this point in all directions, and becomes complete at the antipode on the median side of the nucleus. The alveolar structure of the cytoplasm has itself become finer. The loss of the nuclear membrane allows a slight spread of the chromatin; so that the mass seems to thin out progressively from the center; although a quite definite demarcation between chromatin and cytoplasm always remains. At this time a small aster with its centrosome makes its appearance at a point just outside the spot where the nuclear wall first disappeared. The aster has apparently arisen " de novo " as described by Tennent and Hogue ('06), Scott ('06), and Tharaldsen ('26). The nucleolus fragments and apparently gives rise to chromosomes which can be seen resting free in the nuclear material (Jordan, '08; Wilson, '01). The aster enlarges for a time sending its rays well into the nuclear mass where they become attached to the chromosomes (Fig. 2). The aster together with its centrosome then divides to form the amphiaster of the first polar division (Fig. 3). The first polar spindle is formed in a position tangential to the surface of the egg. When the mitotic figure has reached its maximum size it rotates at right angles to this position, and migrates to the surface of the cell (Fig. 4). Here the chromosomes divide and move to the foci of the two centers (Fig. 5). With the extrusion and pinching off of the first polar body the inner half of the amphiaster with its central body persists for a short time in the outer region of the cytoplasm after the astral rays are lost. The central body soon fades away, leaving a deeply stained area which gradually disappears. The chromosomes migrate a short distance deeper into the cytoplasm of the egg.

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[2] See Tharaldsen, "The origin and nature of cleavage centers in echinoderm eggs." Jour. Exp. Zoöl., V. 44, no. 1.

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On the median side of the scattered chromosomes a small centrosome and central body appears. An aster is formed (Fig. 6) which divides, and the two centers move apart, drawing the chromosomes into an equatorial position. When the asters reach an antipodal position the spindle rotates approximately 90 degrees and migrates to the surface of the egg. Division takes place and the extrusion of the second polar body follows (Fig 7.).

After the extrusion of the second polar body the inner half of the amphiaster together with its centrosome and chromosomes persists for a short time, but soon degenerates as did the first, leaving the chromosomes in a deeply stained area. These give rise to chromosomal vesicles which are drawn together to form the egg nucleus by a monaster (Fig. 8). This condition has been described by Tennent and Hogue ('06) and Tharaldsen ('26). After the vesicular monaster has performed its function it disappears, leaving its center as a darkly stained body at the periphery of the newly formed nuclear membrane. The deeply stained area soon vanishes, followed shortly after by the disappearance of the centrosome. The egg nucleus moves through the cytoplasm to a position nearer the center of the egg (Fig. 9).

At no time in the maturation division are all the chromosomes in the same stage of division, a condition which renders it very difficult to obtain the exact chromosomal count, but never has there been observed in this material more than the haploid number after the extrusion of the two polar bodies. The extrusion of two polar bodies is the usual type of maturation in *Asterias* following artificial activation, and as Tharaldsen ('26) has shown, the only type that will give rise to normal swimming larvæ.

Eggs that Extrude One Polar Body.—In some eggs the first maturation division is completed normally as above described, and the first polar body is extruded. The polar body may be retained under the limiting membrane of the egg in a compressed position, or may be pinched off in the usual manner (Figs. 12–13). The second polar spindle forms normally, but does not rotate in its usual manner, nor migrate to the surface of the egg, but remains submerged in aposition in the immediate proximity of its origin (Fig. 10). Thus, it forms two daughter nuclei which are apparently of equal size (Fig. 11) and possess equal amounts of chromatin material. The nuclei later fuse (Figs. 12–13) to form an unusually large pro-nucleus. During the fusion of the two nuclei an aster makes its appearance near the point of fusion and sends its rays out along the sides of the nuclei (Fig. 12). It may divide to form the amphiaster of the first cleavage spindle, as seen in Fig. 14. A transverse section through the equatorial plate of the first cleavage spindle of such eggs shows clearly more than the haploid number of chromosomes (Fig. 15). In some cases these nuclei may not fuse, but develop monasters that do not divide. These eccentric monasters may complete the first cycle without division and then become secondarily united with a cytaster to form the second polar spindle. This spindle may migrate to the surface of the egg and extrude the second polar body. It is also common here too for the monaster pole of the spindle to divide, giving rise to a tripolar spindle. These tripolar figures in this material have never been observed to extrude the second polar body.

Eggs that Extrude No Polar Bodies.—Some eggs have been observed in which the first polar spindle may apparently become directly the first cleavage amphiaster (Fig. 16). In this case the first polar spindle is formed deep within the cytoplasm near the center of the cell. It is very much larger than the normal, and the ensuing division results in two almost equal blastomeres. A careful search through the egg gives no indication of polar bodies having been formed. Apparently the amphiaster of this figure is the first polar spindle which has become very much enlarged. It seems to form in the usual manner, but instead of migrating to the periphery of the egg, it recedes deeper into the cytoplasm.

It is common also for the first polar amphiaster to form deep within the cytoplasm and divide to form two nuclei similar to those formed by the submerged second polar spindle. The two nuclei have been observed to fuse and give rise to the cleavage nucleus. A monaster arises at the point of contact and by division forms the amphiaster of the first cleavage spindle.

Some eggs have been found where the first polar division is carried through submerged, and gives rise to two nuclei (Fig. 17). In Fig. 18 one of the nuclei has developed asters apparently simultaneously, and is migrating to a central position of the egg. Here it will become directly the first cleavage amphiaster which may divide the egg into two equal blastomeres. I have been unable to trace the fate of the other submerged nucleus, except to observe that it comes to rest in one of the blastomeres. It seems probable, however, that it might develop an aster which would divide to form the cleavage amphiaster of the blastomere.

The two nuclei that are formed by the first submerged polar spindle may develop asters which form amphiasters and divide the two nuclei, forming four nuclei in the egg. These four nuclei have been observed to fuse and form the zygote of the egg.

Monocentric Mitosis.—When a one-aster egg is studied soon after activation, it cannot be determined at that time whether the aster will later become bipolar and give rise to an amphiaster, or will only pass through rhythmic fluctuations that never lead to division. These eccentric monasters pass through growth and recession stages which may or may not be repeated. These cycles correspond in time, roughly, with the formation of the first polar amphiaster, the second polar monaster, and amphiaster of the second polar division. In each cycle the rythmic disappearance and reappearance of the rays and successive division of the chromosomes take place. In Fig. 19 the rays are extended and the chromosomes are scattered in an arc midway the length of the rays. The chromosomes are dividing and will later be recollected at the nucleus where the astral rays are focused. Fig. 20 shows the completion of a monocentric cycle in which the astral rays have been lost and the double number of chromosomes are resting free in the cytoplasm. I have observed eggs where the chromosome count showed they had passed through three monocentric cycles, which were comparable to the first two polar and the first cleavage divisions. In Fig. 21 the first polar division has been carried through normally and the first polar body extruded in the normal way. The second maturation aster has failed to divide and is going through monocentric cycles. The astral rays are receding and the chromosomes have been collected at the nucleus. The astral rays are soon lost to view entirely and the deeply stained zone with a central body persists for a short time; but it, too, is soon lost.

Some Unusual Types.—Many very abnormal conditions of nuclear mitosis arise in eggs that have been over-exposed to the activating agent. Fig. 22 shows an early phase of a tripolar condition in which the asters have taken a triangular position around the chromatin material. This figure is the result of a simultaneous appearance of two centers, such as described by Mead ('98) for the first polar amphiaster in *Chætopterus*. One of the centers has divided and migrated to a position where the force is at equilibrium between the three centers. The other aster has apparently divided, but the central bodies remain close together, forming a wide focus for the astral rays. In some cases the two primary polar centers divide and move apart to form a typical tetraster, as described by Boveri for dispermatic eggs. In such abnormal conditions normal development never follows which is probably due to the abnormal combinations of the chromosomes.

The primary maturation monaster has been observed to divide into two, three, or more centers, giving rise to triasters, tetraster, or multiple asters. These various abnormal astral figures may give rise to several nuclei within the egg. This type of abnormal condition is usually found after a longer exposure to the activating agent.

Fig. 23 is an equatorial plate stage of a tripolar second maturation spindle. The first maturation division has been carried through normally and the astral rays of the distal pole are fading away; while the second polar spindle has united with a cytaster which will divide the chromosomes somewhat unequally among the three centers, thus forming three nuclei with unequal chromatin content.

Fig. 24 shows a telophase of the above tripolar condition. The chromosomes have all been collected at the centers, and are broken down. The asters are beginning to recede and the result is three separate nuclei. These nuclei may or may not fuse. In most cases they have been observed to fuse (Figs. 25–26). In such cases where fusion takes place, all trace of the astral radiations, centrosomes and centrioles, of the three centers are completely lost to view. The chromatin material that has resulted from a fusion of the three nuclei may develop an individual monaster which may never divide; or in some instances they may form an amphiaster which becomes the first cleavage spindle. If the three nuclei do not fuse but remain separate, each nucleus may develop a monaster which forms an amphiaster; this divides the chromatin material again, so that six small nuclei results within the egg (Fig. 29). In another case the two nuclei resulting from the first or second polar mitosis (Figs. 11–17) have formed asters and divided again (Fig. 27) to form four small nuclei, as seen in Fig. 28. In Fig. 28 the four nuclei are in pairs. Each of the two pairs has developed an aster at its point of contact and is fusing to form two large nuclei as in the original case.

Fig. 29 shows six nuclei with asters which are the result of a division of a triaster which forms three nuclei, on each of which an aster has developed. These asters divide, thus bringing about a submerged mitosis, forming three sets of small nuclei without division of the cytosome. These six nuclei may fuse to form three nuclei again, which may in turn fuse to form the cleavage nucleus. Here again the chromosomal count could not be made, due to the many divisions of the chromosomes.

In many eggs several asters and cytasters have been observed.

In Fig. 30 three asters have become attached to chromatin material, forming a triastral condition with two independent cytasters nearby in the cytoplasm. In all cases where cytasters are found associated with nuclear material it is through secondary union. The cytaster is usually much smaller than the nuclear aster, but may vary in size and intensity, as described by Tharaldsen ('26) in *Asterias.* Usually the larger the number of cytasters present the smaller they are in size. Cytasters never divide unless associated with nuclear material; neither do they appear till after the breaking down of the germinal vesicle.

In other cases asters are seen to appear around masses of nuclear material which has failed to vacuolate and fragment in the usual manner. In Fig. 31 a group of asters are present, each of which possesses a portion of the nuclear mass which has failed to be transformed into chromosomes.

The Effect of the Double Method of Activation on the Maturation Divisions.—These observations show that the effects of the two activating agents (butyric acid and Loeb's double method) on the maturation divisions of *Asterias forbesii* initiate similar maturation phenomena within the egg. The first activating agent may cause membrane elevation only, or membrane elevation and monaster formation together with complete maturation, including its abnormal modifications. The second activating agent may resume the activation process and carry it to completion from any point where the astral phenomena initiated by the single agent halted. Either one of the activating agents may be used without the other, or the hypertonic may be followed by the butyric, or their sequence may be reversed. Also both methods give rise to apparently the same type of abnormal phenomena, including eccentric monasters, triasters, multi-asters, and submerged polar divisions. Eggs treated by Loeb's double method, however, give rise to larger polar spindles, more distinct asters, and produce more rigorous maturation phenomena in general than eggs treated by the butyric method alone. A higher per cent of abnormalities in the maturation divisions have been observed to take place after the treatment with Loeb's double method.

It therefore appears from these observations "that from the point of view of astral phenomena, the two agents are not in different categories with respect to their effect on the egg, but they both produce similar results in a continuous process of activation, the second carrying forward the activation effects caused by the first, whatever that may have been."

Discussion.

Cytological studies on the maturation phenomena of artificially activated eggs of *Asterias,* show this process is subject to wide variations in the history of both the chromatic and achromatic figures. It has been shown that maturation may take place with one, two, or no polar bodies extruded. The eccentric monaster is the primary phase of both achromatic figures, which in eggs properly activated may by division give rise to normal amphiaster and lead to the extrusion of each polar body. This process is also subject to wide variation both in number and arrangement of nuclear asters and their secondary union with cytasters.

The evidence in this paper shows the normal type of maturation following artificial activation involves the extrusion of two polar bodies. The dissolution of the nuclear membrane is followed by the appearance of a minute aster in the cytoplasm just outside the remnant of the germinal vesicle at a point nearest the surface of the egg. The aster divides to form the amphiaster which extrudes the first polar body. The inner aster with its central body soon degenerates and is soon lost to view. The second polar spindle is formed by the division of a monaster that arises " de novo " on the median side of the scattered chromosomes. The second polar spindle extrudes the polar body and degenerates as did the first polar spindle. Tennent and Hogue ('06) maintain that this is the only type of maturation that will give rise to normal larvæ.

The second most common type of maturation in artificially activated eggs of *Asterias forbesii* involves the extrusion of only one polar body. The polar spindles are formed as in normal maturation, but the second polar spindle fails to migrate to the surface of the egg and extrude the polar body. Division takes place submerged and forms two nuclei with apparently normal chromatin content. The usual subsequent action of these two nuclei is to fuse and form the cleavage nucleus with the diploid number of chromosomes. This type of maturation has been described by Buchner in *Asterias,* Lefever in *Thalassema,* Allyn in *Chætopterus,* Morris in *Cumingia,* and Brauer in natural parthenogenesis of *Artemia.* These investigators report that apparently normal larvæ may develop from this type of maturation and the embryos possess the diploid number of chromosomes. It appears that normal development might follow this type of maturation in Asterias when the chromatic and the achromatic figures appear normal. The same chance of normal chromosome combination is present as that which exists in fertilization, which is in harmony with the conclusions of Boveri that normal development is dependent on the normal combination of chromosomes. It would, therefore, seem to be quite possible that another type of normal development following artificial activation may take place in *Asterias* which has a diploid number of chromosomes present.

In few cases no polar bodies are extruded following artificial activation. The first polar spindle may become directly the first cleavage amphiaster or the first polar amphiaster may divide submerged and form two nuclei which fuse to form the cleavage nucleus. Tharaldsen states he has never observed normal development following such abnormal conditions, which is probably due to the abnormal chromosome combinations.

In some cases the maturation monasters do not divide but pass through definite cycles of activity involving the rhythmic disappearance and reappearance of the rays and successive divisions of the chromosomes without division of the aster or the cytosome. Wilson ('01) has described five successive cycles of the monaster which correspond to the two maturation divisions and the first three cleavage divisions. In eccentric monaster eggs, at the telophase of the cycle, the chromosomes collect at the focus of the rays as the astral radiations disappear and give rise to a nucleus with many chromosomes. Such eggs never cleave and activity soon halts, followed by the degeneration of the monaster.

The origin and nature of the central bodies in maturation are similar to that described by King ('06) and Tharaldsen ('26) in *Asterias.* The monaster of the first polar division appears "de novo" just outside of the remnant of the germinal vesicle in the cytoplasm. It divides to form an amphiaster which extrudes the

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first polar body. Mathews ('01) claims to have been able to trace the origin of the centrosome from a small granule (which divides into two bodies before passing out of the germinal vesicle) within the nuclear membrane, and is of the opinion that these bodies migrate to the periphery of the chromatic material and become the centers of the first polar division. Careful search through the chromatin material in *Asterias* before maturation sets in shows no structure that might be comparable to that described by Mathews. If there are such structures they are indistinguishable from the other granules of the cytoplasm. In no case has it been possible to identify by the usual criteria small, dark-stained bodies that give the appearance of a centrosome in the cytoplasm of the egg. Mathews has described the rupture of the nuclear wall where the central bodies pass from the chromatin material into the cytoplasm. These experiments show no evidence of such a condition existing in *Asterias*.

In the formation of the second polar spindle the evidences in this paper agree with Tennent and Hogue ('06), and King ('06), that the amphiaster is formed by the division of a monaster that has arisen " de novo " on the median side of the scattered chromosomes. The second polar spindle rests tangential to the periphery of the egg until it has reached its normal size and the chromosomes are pulled into the equatorial plate. The spindle then rotates and migrates to the periphery of the egg and extrudes the second polar body. Mead ('98) and Jordan ('08) believe they have definitely observed the outer aster of the second polar division to appear at the old-mid-body in the position of the old equatorial plate of the first division. The second polar spindle has been definitely observed in this material to be formed by the division of the secondary monaster and comes to lie in a position tangential to the periphery of the egg. No trace of a mid-body could be found as described by Mead and Jordan after the disappearance of the first polar spindle. Therefore, these evidences show that the contentions of Mead and Jordan do not apply to this material.

Buchner has described the centrosome of the second polar spindle to persist and give rise to the first cleavage amphiaster. He contends that the inner centrosome of the second maturation division, after the astral rays have been lost, follows the pronucleus to its position near the center of the cell. This evidence supports the views of Van Beneden and Boveri that the centrosome is a permanent cell organ handed on by division from one cell to the other. I have been unable to find in this material evidence which shows central bodies or astral rays accompanying the chromosomes to their position of rest near the center of the cell, after the second polar body has been extruded.

The evidence in this paper shows that the central bodies of the achromatic figure are not a genetically continuous structure, but arise " de novo " as shown by their appearance and disappearance with each polar division. This is further illustrated by the fact that in abnormal conditions two centers may arise simultaneously to form the centers for the first or second polar spindle. In very abnormal conditions a great many such centers may arise simultaneously; a condition which precludes the possibility of a genetic continuity between centers. Therefore, the contentions of Boveri, Buchner and Mathews of the genetical continuity of central bodies are not tenable for maturation in *Asterias.*

The achromatic figures may be distorted and further complicated by secondarily uniting with cytasters. Such secondary union with cytasters may give rise to amphiasters, triasters, or a multi-asteral condition. It is impossible for such types to give rise to normal embryos. Boveri in his experiments with dispermic eggs has long since demonstrated the qualitative difference in chromosomes and the necessity of their proper combination. It would, therefore, be apparent that equivalent and fully potent daughter nuclei will result only from regular nuclear division with two centrosomes; and that when three or more controsomes are present the single daughter nuclei will not contain the full number, or proper combination of the chromosomes, and as a rule, not qualitatively the same nuclear material. Thus, the chance for normal chromosome combination in triasters and multi-asters is far less probable than in the amphiaster condition.

These observations have shown that the single and the double method of activation initiate similar maturation phenomena in *Asterias forbesii.* The double method does not produce any condition or in any way change the mechanics of the maturation process from that observed in the single method. Either agent of the double method may be used alone, and, if given proper exposure, will produce the same effect. The second agent therefore simply accelerates the activation initiated by the first. There may be all degrees of activation and resultant astral response by either one or both the agents employed. Therefore, Herlant's and Herbst's contentions that in the double method the first activating agent produces a monaster, and the second agent produces a cytaster which unites with the monaster to form either of the polar spindles, is untenable. This contention not only does not agree with observed conditions in *Asterias,* but is totally unlike anything occuring in the normal process; and it has been shown that, following artificial activation, maturation must proceed normally in order that normal development may result.

Conclusions.

1. Maturation in artificially activated eggs of *Asterias* may involve the extrusion of one, two, or no polar bodies, but only eggs that extrude two polar bodies apparently develop normally. It seems possible that eggs extruding one polar body might develop normally but it has not been demonstrated in this material.

2. These observations have shown that eggs artificially activated by (1) butyric alone, and (2) butyric acid followed by hypertonic sea-water, are affected similarly in their maturation processes. The second agent in the activation process seems to intensify the stimulus of the first.

3. Cytasters apparently arise " de novo."

4. The primary phase of each maturation amphiaster is a monaster which arises " de novo " and subsequently divides to form the dicentric figure.

5. The extrusion of two polar bodies, which has been shown to be the normal method of maturation under artificial activation, results in the presence of a haploid number of chromosomes within the embryo which develop normally. The normal diploid number of chromosomes can only be obtained by the method whereby the second polar body fails to be extruded, which may possibly give rise to normal embryos.

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Plate I.

Explanation of Figures.

All drawings were made from camera lucida sketches at table level, with 43 × objective and 10 × eyepiece. Drawings reduced approximately one-half in reproduction.

Fig. 1. Primary oöcyte.

Fig. 2. First polar monaster before division.

Fig. 3. Division of the first polar monaster.

Fig. 4. First polar amphiaster before extrusion of first polar body.

Fig. 5. Late anaphase of the first polar amphiaster.

Fig. 6. Second polar monaster before division.

Fig. 7. Anaphase of the second polar spindle.

Fig. 8. Vesicular monaster.

Fig. 9. Egg after completion of maturation.

Fig. 10. Late anaphase of a submerged second polar spindle.

Fig. 11. Two submerged nuclei of the second maturation division.

Fig. 12. Start of fusion of the two submerged nuclei of the second polar division. An aster has made its appearance near the point of contact.

Fig. 13. Advanced stage of the formation of zygote by fusion of two submerged nuclei formed from division of submerged second polar spindle.

Fig. 14. Early amphiaster of first cleavage spindle in which the zygote had been formed by fusion of two submerged nuclei of the second polar division.

Fig. 15. Transverse section through the equatorial plate of first cleavage spindle of an egg in which the zygote had been formed by fusion of two submerged nuclei.

Fig. 16. First cleavage amphiaster that has been formed directly without extrusion of polar bodies.

PLATE I.

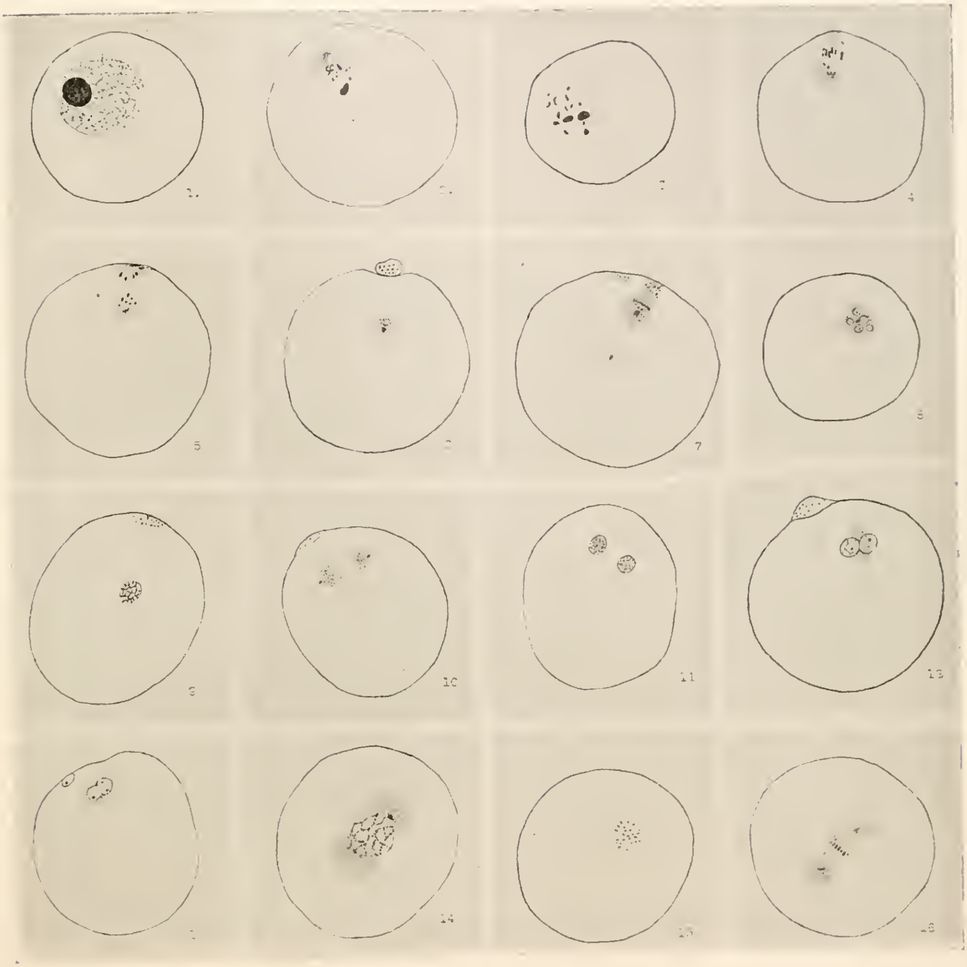
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PLATE II.

FIG. 17. Two nuclei formed by a submerged first polar spindle.

FIG. 18. Early first cleavage amphiaster formed from a submerged nucleus of the first polar spindle.

FIG. 19. Eccentric monaster first polar division.

FIG. 20. Resting stage of a second polar monaster that shows approximately 50 small chromosomes.

FIG. 21. First cleavage concentric monaster.

FIG. 22. Prophase triaster first polar division.

FIG. 23. Triaster second polar division.

FIG. 24. Late telophase triaster first polar division.

FIG. 25. Fusion of three nuclei formed by triaster of second polar division.

FIG. 26. Fusion of three nuclei that was formed from triaster of first polar division.

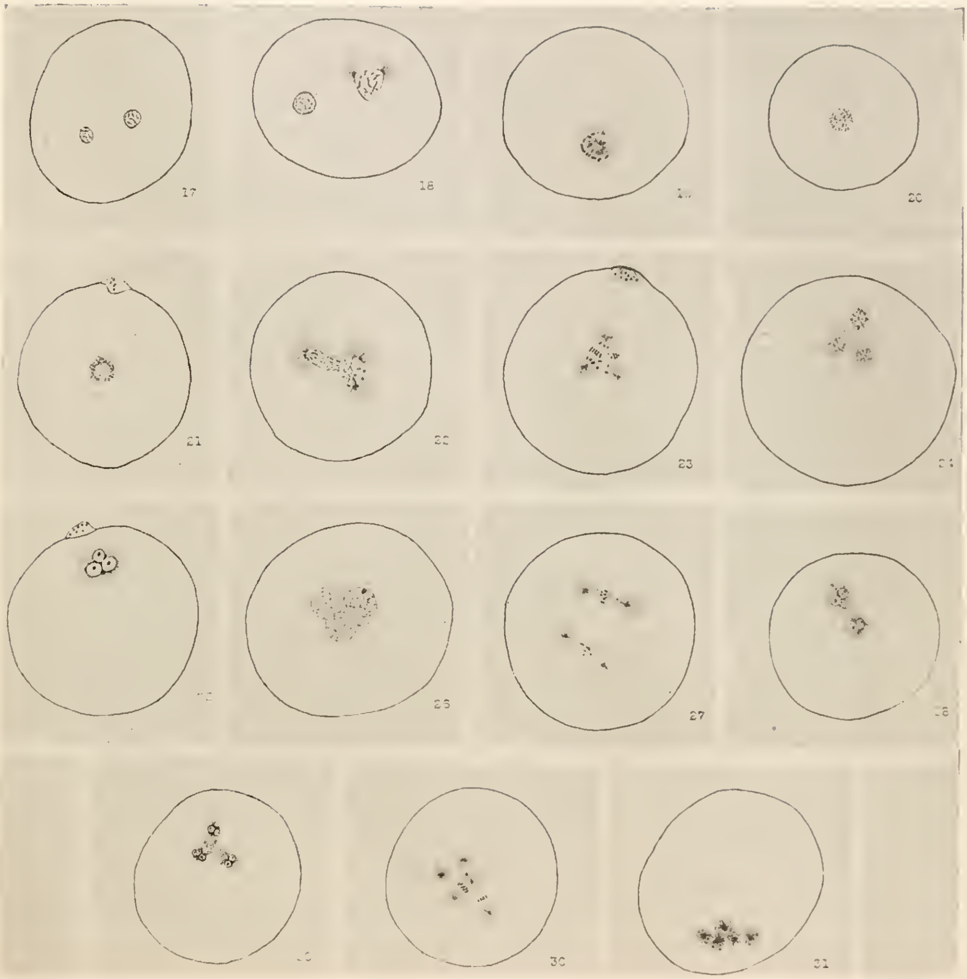
FIG. 27. Amphiasters of two submerged nuclei formed from the first or second submerged polar divisions.

FIG. 28. Four nuclei formed from division of two submerged nuclei.

FIG. 29. Six nuclei formed from division of three submerged nuclei.

FIG. 30. Triaster and cytasters.

FIG. 31. Asters formed around masses of chromatin material.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H. W. BEAMS.

THE CROSS FERTILIZATION OF ENUCLEATED *ECHINARACHNIUS* EGGS BY *ARBACIA* SPERM.

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If a pluteus can be produced when an enucleated egg of one Echinoderm is activated by a sperm of another species having different larval characters, information may be secured concerning the respective rôles of chromatin and cytoplasm in heredity and development.

Investigations of this problem down to 1924 have been reviewed by Morgan ('24). Boveri's early experiments ('89, '95, '14) with *Sphærechinus* eggs activated by *Echinus* sperm are invalidated by uncertain enucleation, as pointed out later by Boveri himself ('18). When eggs are shaken to pieces and supposedly enucleated fragments are picked out, he found that if they are studied in section some of them are seen to contain chromatin undetectable in the living condition. Since he obtained enucleated eggs by the shaking method, the larvæ produced do not yield dependable evidence due to the possible presence of some maternal chromatin. Morgan ('96) working with the same species attempted to activate enucleated eggs of both of them with sperm of the other, but there was no development. Godlewski ('06) secured four maternal gastrulæ when enucleated *Echinus* eggs were activated with *Antedon* sperm, but his work, like Boveri's, is invalidated by the fact that the supposedly enucleated eggs, obtained by the shaking method, probably contained undetected maternal chromatin.

Taylor and Tennent ('24) satisfied the requirement of unquestioned enucleation by cutting eggs singly with a microdissection apparatus. Of forty-nine enucleated eggs of *Lytechinus* inseminated by *Tripneustes* sperm fifteen cleaved and one formed a blastula without mesenchyme. In the reverse cross only membranes were formed and there was no cleavage. They note that their investigation is not conclusive since *Tripneustes* was not in good condition.

In the present investigation[1] 689 enucleated eggs of *Echinarachnius* inseminated by *Arbacia* sperm produced forty-six blastulæ. Since the embryos died at about twelve hours, before maternal and paternal characteristics could be distinguished, a critical analysis concerning nuclear and cytoplasmic inheritance was not secured. Certain observations were made, however, a report of which may be of value to future investigations.

Dry eggs freshly obtained from the gonopores (Just, '19) do not give a higher percentage of activation, after enucleation, than eggs freshly obtained from the surface of the ovaries.

Eggs were enucleated one at a time by a glass needle held in the hand (Fry, '24). The cutting is done on an ordinary microscope slide placed on the stage of a dissecting microscope. This permits more rapid enucleation than can be accomplished with the machine as about twenty eggs can be enucleated in ten minutes. Hence it is possible to experiment with relatively large numbers of enucleated eggs rather than with several at a time, thus increasing the chance of securing a significant result. That the method gives sure and certain enucleation was shown in an earlier study (Fry, '25).

Enucleated eggs of *Echinarachnius* are viable only when fragmented at temperatures between 15° C. and 20° C. If the cutting is performed at temperatures either below 15° C. or above 20° C., there is practically no activation. The microscope is placed in a box having a glass top through which the oculars project. Openings in the front permit the entrance of the hands. The temperature is roughly controlled by ice containers. Since the air within the box is thoroughly moist, this reduces evaporation from the drop of water on the slide that contains the eggs and fragments. Thus changes in osmotic pressure are reduced to a minimum.

No effort was made to locate the micropyle by the use of inks and the eggs were cut by chance in any plane. Therefore, no data were secured as to the relation between cutting in different planes and the resultant effects upon the development of enucleated fragments.

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[1] The experiments were carried on at the Marine Biological Laboratory at Woods Hole, Massachusetts, during June and July 1926.

The majority of the enucleated eggs were about three fourths the size of a whole one. None were less than one half size and many were nine tenths size or even larger since every effort was made to remove the nucleus with a minimum amount of cytoplasm. Within these size limits, from one half to nine tenths, there is no correlation between the size and the capacity to respond to activation by *Arbacia* sperm. This indicates that failure to secure development in such experiments is not due to the size of the fragments if they be at least half the size of a whole egg. The lowest size limit capable of activation was not ascertained.

Just ('19) found that the best method of activating *Echinarachnius* eggs with *Arbacia* sperm is by the use of a heavy sperm suspension. In the present experiment with enucleated eggs various dilutions of sperm suspension yielded about the same percentages of activation, so long as the suspension was at least three drops of seminal fluid to 50 cc. of sea water. Heavier suspensions do not modify the amount of development.

Whether the enucleated eggs are activated a few minutes after cutting, or as late as an hour afterwards, the percentage of development is the same. A period of recovery from so-called "shock" is not required.

The 689 enucleated eggs yielded forty six blastulæ, giving about six per cent. development. Whole *Echinarachnius* eggs when similarly activated with *Arbacia* sperm yield an average of eighteen per cent. development (Just, '19). This difference in percentage of development is probably a result of the general maltreatment of enucleation.

It would be expected that there might be a considerable amount of irregular development among enucleated eggs caused by polyspermy. In most cases, however, either the enucleated eggs cleave regularly or they do not cleave at all, and there is little irregular cleavage. Although polyspermy and consequent irregular cleavage occur in fragments obtained by shaking eggs to pieces (Morgan, '96), polyspermy, as judged by irregular cleavage, is rare in fragments obtained by cutting with a glass needle. The shaking method is probably quite harmful to the egg and wrecks its mechanism for controlling polyspermy, whereas the glass hair leaves it in a more normal condition.

Embryos were reared in 5 cc. round-bottom salt cellars under the conditions described by Tennent ('10). Great care was taken to use sterilized sea water, to change it several times a day, to prevent evaporation, and to keep it at the temperature of the running sea water in the aquaria.

The blastulæ appeared quite regular but were more frail and delicate than normal ones. Most of them died at about twelve hours. Mesenchyme cells were not observed. Five blastulæ lived about two days; they were top swimmers, but became opaque and underwent no invagination.

In his final work Boveri ('18) came to the conclusion that completely enucleated eggs of *Sphærechinus*, as identified by the small size of the haploid nuclei, when activated by *Echinus* sperm rarely produce a gastrula and seldom, if ever, produce a pluteus. Taylor and Tennent ('24) secured but one blastula, and it did not gastrulate, in the cross between enucleated *Lytechnius* eggs and *Tripneustes* sperm. The present experiment yields a similar result since the forty-six blastulæ failed to gastrulate.

Enucleated eggs of *Echinarachnius* when activated by *Arbacia* sperm produce blastulæ that do not gastrulate whereas whole eggs similarly activated produce gastrulæ and plutei. This difference in development between enucleated eggs and whole ones when cross fertilized might be attributed to the general harmful effects of enucleation involving general maltreatment and the loss of some cytoplasm. This is probably not the cause, however, since the enucleated eggs are in a sufficiently normal condition to be activated by the sperm, to effect cleavage, and to form blastulæ. The probable explanation of the difference in development lies in the fact that the whole eggs contain a haploid set of their own chromosomes that normally function in that cytoplasm, in addition to the other set of another species introduced by the sperm, whereas the enucleated eggs contain only the chromosomes of another species functioning in an alien cytoplasm. The experiment demands that the two species used shall not be too closely related, for they must be sufficiently separated to have different pluteus characteristics in order that the respective rôles of maternal cytoplasm and paternal chromatin may be analyzed by those very differences. It is probably a significant comparison that *if an Echinarachnius egg is activated by the sperm of a somewhat distantly related species, development goes through to pluteus formation if the maternal chromosomes are present, but does not go beyond the blastula stage if the maternal chromosomes are absent.* The exact meaning of this involves an explanation of the interaction of the various factors concerned at various stages of development in whole eggs and enucleated ones, *i.e.*, the maternal cytoplasm into which was released maternal nuclear substances at maturation; the later effects of maternal genes; and the later effects of genes of another species. It is such an analysis of these factors that this type of experiment is designed to effect, but the failure of the embryos from enucleated eggs to develop beyond a blastula prevents an analysis of the problem. Whether or not a gastrula and pluteus can develop from an enucleated egg of one species when activated by the sperm of another species, that is sufficiently distant in relationship to have different larval characters, awaits the results of further experiments on various forms.

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A PRELIMINARY REPORT ON THE STRUCTURAL ELEMENTS OF THE CYTOPLASM IN PLANT CELLS.

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One Plate.

In several recent papers (Bowen, '26*a*, *b*, *c*) I have reported briefly on some aspects of cytoplasmic structure in plant cells, as revealed in the first stages of an extensive study of the problem as a whole. This investigation has now been pushed considerably farther and much more complete results are in hand. Errors in my earlier reports, both of omission and commission, have now become evident, and I propose in this paper to restate the major results by way of correcting and extending my previous accounts, pending the completion of more extended papers, now in preparation.

Material.

My study thus far embraces material from each of the three higher plant groups, Bryophyta, Pteridophyta and Spermatophyta, as follows:

Bryophyta—male heads (including developing sperm cells, antheridial epithelium, and cells of the paraphyses and leaves) of *Polytrichum juniperinum*, *piliferum* and *commune*.

Pteridophyta—growing-point and root-tip of *Equisetum arvense*.

Spermatophyta—young shoots of barley and root-tips of *Vicia*, *Pisum*, *Ricinus*, *Hyacinthus*, barley, pumpkin and kidney bean.

Some of this material has received as yet only preliminary study, but so far as the results go, there appears to be a surprising uniformity in plant cells of all kinds as regards the general morphology of the formed elements of their cytoplasm.

Methods.

The problem of *how* to study plant cells is one which seems to have received astonishingly little attention. The most extensive work in recent years on plant cytoplasm is undoubtedly that of Guilliermond, who arrives at the conclusion that the fixatives involving osmic acid, so long used by zoölogists, give very erratic and misleading results on plant tissue and are therefore in general to be avoided. He himself and his students employ a method which is essentially one of Regaud's formulæ, and which in Guilliermond's laboratory seems to have yielded excellent results. I have given this method extensive trial on root-tips with most unhappy results, and have finally discarded it completely as unusable in my hands. The details of the method have never been published very fully, and presumably I have made some unavoidable technical errors which had fatal results. In place of this method I have concentrated on the methods long used by zoölogists in the study of animal cytoplasm, and can not agree with Guilliermond's conclusion as to their lack of value for the study of plant cells. On the contrary, I find that with proper experience and controls these methods yield results on plant cells of a value in every way equal to those obtained in animals, and in some respects superior to Guilliermond's method as successfully employed by himself. Quite a number of different methods have been tried, but the following have proved most generally useful:

Benda—using Mottier's ('18) modification of the fixative, and continuing as for the Benda method with animal tissues. This method stains the plastidome more or less specifically in meristem cells, and is very useful for separating it from other components.

Champy-Kull—following the directions exactly as given for animal tissue. This method stains the pseudochondriome (see beyond) more or less specifically and in some cases will separate it completely from all other components of the cytoplasm.

Kolatchev and *Weigl*—following the directions generally given for animal tissues, but with some slight modifications suggested by experience. These methods were first employed by me for an extensive study of plant cells, the results having been briefly published nearly a year ago (Bowen, '26*a*). Meanwhile Guilliermond ('26) had been independently working with the Kolatchev and other osmic methods and his results, published shortly after my report, corroborate my findings in a general way, but offer some very remarkable differences in detail. My work with these methods has shown that they will impregnate practically everything in a plant cell except chromosomes (and probably central bodies when they occur). The classes of materials thus demonstrated may be tabulated as follows:

1. Osmiophilic platelets,
2. Plastidome,
3. Pseudochondriome,
4. Vacuome,
5. Nucleus—not including the nucleolus as a rule,
6. Intra-nucleolar bodies,
7. Spindle fibers, cytoplasmic network and cell plate,
8. Intra-vacuolar masses.

The outcome of any particular trial can not in general be foretold, and these osmic methods can only be interpreted with accuracy after extensive experience—and not always then. In spite of the obvious disadvantages arising from their excessive lack of specificity, these methods nevertheless yield results unequalled by any other technique, and can not fail to be of extraordinary interest and value to anyone engaged in the study of plant cells.

The methods here outlined together with others will be given in another paper the detailed consideration which space does not now permit.

Results on Root-tips.

The Osmiophilic Platelets.—Following osmic impregnation, the most generally demonstrated components of the cell are the osmiophilic platelets. I have given them this name merely as a convenient descriptive term, pending the final adjudication of their status. They have the shape of plates or discs, the periphery of which is intensely blackened by osmic acid (Figs. 1 and 8). The central part may be quite clear or may be slightly blackened or browned in varying degree. Just what the exact morphology of these bodies may be is not known, but the impression one gets is that of a plaque with a peripheral rim of specifically different composition. When seen in face view, the blackened rim appears as a ring, while in profile it appears as a rod, ellipses of varying size being apparently seen at intermediate angles of vision. These platelets have now been demonstrated with the greatest clearness in practically all the tissues mentioned in this paper, and throughout the three higher plant groups they appear to be a constant and characteristic part of the cytoplasm. They are usually very numerous, sometimes running into the hundreds, as in the large cells of the barley root-tip. In general they are rather small (Fig. 1) but they tend to be larger in the root-cap cells of spermatophytes, and less numerous and more conspicuous in the androgonia of *Polytrichum* (Fig. 8). They show no peculiarities of distribution in either the resting or dividing cell, except possibly a tendency to heap up along the cell plate in the telophase of division. They seem to multiply by a process of fragmentation, but nothing very definite can be said on this point. Thus far they do not appear to have any function capable of expression in terms of their visible morphology, except in the moss androcyte.

The most extraordinary thing about these platelets is that they are so readily demonstrable by the methods used for the animal Golgi apparatus, but, with rare exceptions of a special nature, can not be demonstrated by any of the usual staining methods. It appears probable that these bodies as a permanent and generalized component of plant cytoplasm have never been described before, indeed may very probably never have been seen by botanists. It is an extraordinary fact that Guilliermond ('26) does not mention these platelets in his recent paper, and the only explanation I can offer is that they have possibly been mistaken for one of the other and well-known cytoplasmic constituents. He has, in an earlier paper ('24), claimed that plant cells in general contain lipoidal granules which he calls the "granulations lipoides." These are granules, not platelets, and they are easily demonstrated by osmic methods which do not suffice for the osmiophilic platelets. I am, therefore, of the opinion that these fatty granules of Guilliermond represent exactly what he calls them, and that they have nothing to do with the osmiophilic platelets here described. The lipoidal granulations would appear to correspond roughly to the fatty granules or droplets occurring in many animal cells, and they have in all probability only secondary interest for purposes of a generalized understanding of cytoplasmic structure. In my first two reports ('26*a*, *b*) I called these platelets the "spherosomes" after the terminology of the Dangeards, and at first suggested their homology with the "inactive chondriome" of Guilliermond. Extended study of other classes of formed bodies in the plant cell has, however, convinced me that such an identification is impossible. These platelets are something new, and certainly have nothing to do with Guilliermond's chondriosomal granules, as will appear more clearly in a succeeding section.

There is, finally, nothing about these platelets in root-tip cells to furnish a critical demonstration of their status in the plant cell. Their regular response to the Golgi apparatus methods and their striking similarity to the scattered Golgi bodies of many animal cells, suggests their homology with the animal Golgi apparatus. Indeed, as will be indicated later, this is probably more than a mere matter of coincidence, but for the moment I wish merely to emphasize the lack of any conclusive evidence as to their nature so far as such evidence might be furnished by observations on root-tips.

The Plastidome.—This comprises a group of formed bodies which occur generally in plant cells, appearing in older meristem and more differentiated plant cells as centers for starch production, deposit or storage—the familiar plastids of various kinds. In the earliest meristem, the forerunners of the plastids are already present and may be called, in general, proplastids. I have found thus far no evidence whatever in root-tips that the plastidome ever has any relation to other cellular components, and everything indicates that the proplastids constitute an independent group of materials whose sole function is the production of plastids in the more differentiated cells of the plant. In root-tip cells the proplastids possess in general an elongate, thread or rod-like shape (Figs. 2 and 3). They may be blackened by osmic acid, especially in *Vicia*, but are probably best demonstrated in Benda preparations, occasionally, as in the hyacinth, with acid fuchsin. Every step in the gradual dep·

osition of starch in the plastid primordia has now been traced, especially in cells of the root-cap and periblem, and their transformation into typical plastids is undoubted. My results thus confirm the morphological results obtained by Guilliermond, who has particularly emphasized as characteristic the generally elongate shape of the proplastids in all sorts of meristems.

The plastidome is characterized further by its general tendency to be oriented in a specific way with respect to the spindle of a dividing cell. The facts have been brought out particularly by Nassonov ('18), and my results corroborate his account, and push the matter somewhat further. In Fig. 2, an especially fine prophase example of the orientation of the proplastids with respect to the future spindle is shown. It now seems more and more probable that this behavior of the plastidome in cell division is a very common phenomenon in plant cell-division, and has been long overlooked simply because of the technical difficulties which so often bring disaster at the particularly important moments of division.

Guilliermond has claimed that the plastidome is a specially differentiated part of the chondriome, characteristic of plant cells. My observations on root-tip cells do not offer any evidence in support of such an identification. Certainly these bodies offer distinct differences in staining capacity as compared to any other cellular elements in plants, and it does not seem possible to settle their status on the basis of their behavior in root-tips.

The Pseudochondriome.—This comprises a group of bodies to which Guilliermond has given the name of "inactive chondriome," in contrast to his "active chondriome" or plastidome. There are in fact several good reasons for believing that these bodies are equivalent to the chondriosomes of animal cells, but pending more complete evidence I have chosen to use the term pseudochondriome as descriptive of their similarity to chondriosomes, but without committing myself for the present to any definite view of their real nature. In my first reports these bodies were confused with the osmiophilic platelets, because of certain morphological similarities when representatives of the two classes are viewed in the proper plane. Indeed, several people upon seeing preparations of the osmiophilic platelets and of the pseudochondriome have asked upon what basis any separation can be made. It is true that the one class of bodies is composed of (flat) discs, the other of spherical granules, but it was thought that possibly the morphological differences were due mainly to the different technical treatment. The first suspicion in my own mind of the distinction between these two components came from a study of the moss androgonia, and I eventually spent several months during the past fall trying to get critical evidence on this point. This evidence I believe is now conclusive, and the pseudochondriome and osmiophilic platelets must be considered as entirely distinct kinds of bodies.

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The pseudochondriome consists of a larger or smaller number of granules scattered throughout the cell and showing no important relations to the spindle in cell division (in root-tips at least). These bodies rarely blacken with osmic acid, an exception being the hyacinth, where they are finely demonstrated as jet black granules on a clear background (Fig. 3). They are occasionally stained by Benda's method or Fe-hematoxylin, but by far the best method is that of Kull. In good Kull preparations (Fig. 4) each granule shows a typically vesicular structure, with a brightly stained periphery and a light center—a composition quite common in the true chondriosomes of molluscan and lepidopteran sperm cells, for example. In older cells these granules often show signs of elongation, etc., but their typical form in the earliest meristem is that of vesicular granules. My results accordingly agree in a general way with Guilliermond's findings, and the facts also indicate that his interpretation of these bodies as chondriosomes is also correct. But it must again be emphasized that there is nothing in root-tip cells themselves upon which to base such a decisive conclusion as to their homologies with the animal chondriome.

Finally as to the distinction which I have drawn between these bodies and the osmiophilic platelets, three lines of supporting evidence combine, it seems to me, to render it secure. In the first place, there are the obvious differences in morphology. The pseudochondriosomes are spherical, and thus, while presenting the appearance after proper staining of rings, these optical sections are always rings, while in case of the osmiophilic platelets, the ring appearance holds from only one point of view, the plates appearing as rods or ellipses when seen on edge or obliquely. In the second place, in the hyacinth the plastidome, pseudochondriome and osmiophilic platelets can all be demonstrated in the same cell with osmic acid, and thus the distinction between all three classes can be demonstrated (Fig. 3). Finally, after blackening the osmiophilic platelets, it is sometimes possible to counterstain with acid fuchsin, thus demonstrating the platelets in black and the pseudochondriosomes in red in the same cell.

The Vacuome.—In many kinds of cells I have found that the vacuome can be impregnated with osmic acid, and thus permanent preparations of great brilliance can be secured. Such preparations have obvious advantages over the vital-staining methods usually employed heretofore. Since the fact that various methods designed for the demonstration of the animal Golgi apparatus have been used with some success for demonstrating the vacuome in plants, and since at some stages the plant vacuome may assume net-like appearances, it has been claimed by Guilliermond and others that the vacuome represents the Golgi apparatus in animal cells. Quite apart from the ultimate merits of such a claim, I wish to draw attention to certain features presented by the vacuome after osmic treatment, which at least suggest that the response of the vacuome to this technical treatment may have little or nothing in common with the blackening of true Golgi material.

There is, in the first place, a distinct difference between the action of the Weigl and of the Kolatchev methods. One may work very much better than the other on a particular kind of cell. Furthermore, the vacuome of some kinds of cells responds regularly, of others irregularly, and of still others not at all, to the same osmic treatment. In addition the type of response differs. Sometimes the vacuoles as a whole (Figs. 5 and 7) are intensely and evenly blackened; in other cases only more or less well-marked traceries of black or gray mark the vacuoles (Fig. 6), these traceries running perhaps through the substance of the vacuole as well as over its surface. Thus the suspicion is aroused that the reaction to osmic acid is largely a response of the contents of the vacuole to osmic acid, the contents responding positively or negatively according to its composition. Thus some root-tips will respond, while in other plants the results are negative or very indifferent. This should certainly not be the case if the vacuome as a whole represents the Golgi apparatus, and the conclusion certainly seems justified, that the claims so far made are not in agreement with the known behavior of osmic acid toward the animal Golgi apparatus. In a preceding report (Bowen, '26*b*) I inclined to the belief that the vacuolar primordia had lipoidal affinities, possibly in the sense that the vacuolar walls were lipoidal in nature. Such a view still seems to me a possible one, and it would certainly make easier an explanation of the results as a whole—but whether or not such an enveloping lipoid membrane occurs, can not now be finally settled. Should such a membrane occur, it might be possible to relate it to the Golgi apparatus, but as to the contents of the vacuole, no relation with the animal Golgi apparatus itself seems possible.

The most complete results on the morphology of the vacuome have been obtained on the root-tips of *Vicia* and *Ricinus*, particularly the former. In the earliest meristem cells the cytoplasm is crowded with many small, more or less spherical vacuoles or vacuole primordia (compare Fig. 5, an older stage in *Ricinus*). In *Vicia* these vacuole primordia undergo rather different histories in plerome and periblem. In the plerome, the small vacuoles of the earliest stages gradually merge together (Fig. 6), growing larger meanwhile, until the large vacuole characteristic of plant cells emerges. All phases in the fusion can be impregnated with unusual clarity in the periblem of *Ricinus*. In the periblem of *Vicia* a remarkable network is developed by transformation and fusion of the vacuole primordia (Fig. 7), later passing on into the large single-vacuole condition. In cell division (Fig. 7, left-hand cell, prophase), these networks are frequently more or less fragmented, presenting a very characteristic appearance. It is claimed by some who have seen my preparations that such bizarre networks can not possibly be normal, but the results of the Dangeards and particularly of

Pensa ('13) on living cells indicate that these osmic impregnations are really remarkable demonstrations of the actual condition of the vacuoles. This is borne out by the fact that such networks in the periblem are characteristic only of *Vicia* (among the forms so far studied by me).

My results on the vacuoles thus bear out the recent extended researches of the Dangeards and of Guilliermond, and go far toward establishing the vacuome as an independent and permanent part of the plant cell system.

Other Cell Structures.—Of the other constituents mentioned in an earlier section as being blackened by osmic acid, none throw any light upon the general problem of cytoplasmic components, and they will not therefore receive further mention here. My results on the blackening of the spindle fibers corroborate, in the main, those of Nassonov ('18), to whose findings I have added the interesting fact that the cell plate can be blackened at all stages of its formation.

Antheridia of Polytrichum.

The results of my study of root-tips are now fairly complete, and they lead unmistakably to the conclusion that the cytoplasm of plant cells contains four classes of formed elements, which are apparently distinct and independent, and probably self-perpetuating under normal circumstances. In animal cells there are only two such generalized classes of formed bodies—chondriome and Golgi apparatus—at least so far as known at present. It is thus impossible to draw any off-hand conclusions as to what components in the plant cell are equivalent to those of the animal cell. Furthermore, the study of meristem cells in root-tips gives no conclusive answer to the problem of homologies. As a means of getting around this unhappy impasse, I suggested in a previous note (Bowen, '26*b*) the possibility of finding processes in the formation of plant sperms which would be comparable to those now known to occur universally in differentiating animal sperms. I have already reported briefly on the probability of achieving success in this direction. Further study, particularly of more successful osmic impregnation preparations, has, however, clearly indicated one or two errors

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in my first account. These I wish now to correct, leaving several further points undecided pending additional study of the material as a whole. I have examined thus far the antheridia of three species of *Polytrichum—juniperinum*, *piliferum* and *commune*. While some minor differences doubtless occur, for practical purposes these three species may be considered as a unit basis for description.

In the androgonia and androcytes the vacuome has not yet been identified, and it is uncertain whether any occurs. The three other cytoplasmic components occur in clearly developed form. The osmiophilic platelets (Fig. 8) are similar to those in the root-tips. They tend to become smaller as the size of the multiplying androgonia diminishes, and are distributed apparently intact to the daughter cells of each division, and without any obvious orientation with respect to the spindle. They arrive in the androcytes as scattered bodies of the same shape and staining capacity as in the androgonia. The pseudochondriome is represented in early androgonia by scattered vesicular granules (Fig. 10), exactly similar to those in root-tips. In later generations of androgonia they frequently undergo changes in shape, becoming rod- or thread-like. They seem to arrive in this form in the androcyte, although on this point I am still in some uncertainty. In cell division they show relations to the spindle axis of several types which can not here be elaborated upon. The plastidome occurs in the earlier androgonia in the form of an extensive lamella or plate (Fig. 10), the polar plates of Allen ('17). In later generations these become more compact and are molded into definite rings, of which one occurs in each cell. Fig. 9 shows an antheridial tip with the epithelium enclosing the central mass of androgonia. The plastids in the epithelium and the polar plates or rings are clearly blackened by osmic acid, yielding a remarkably clear preparation. A study of young antheridia in all stages of formation indicates pretty clearly that these polar plates are directly descended from the plastids of the cells which formed the primordia of each antheridium. In the developing male cells their identity is retained but their function as starch-producing plastids is apparently suspended. They have a striking point in common with the plastidome of root-tip cells, viz., their orientation at the spindle poles during cell division, as observed by Allen. With the spindle itself, however, I can not find that they have anything to do. I have followed all stages in the division of rings like those of Fig. 9, preparatory to cell division, but I have not been able as yet to verify Allen's account of their fragmentation in the older generations of androgonia. The mode of their distribution to, or their morphology in, the androcytes has not yet been certainly made out. In general it may be said that the plastidome of moss androgonia shows an essential similarity to that of the ordinary meristem cells of spermatophyte root-tips.

It remains now to follow the history of these various components in the androcytes as they gradually transform into sperm cells. The history of the osmiophilic platelets (called spherosomes in an earlier report (Bowen, '26*b*)) will first be indicated. In the early androcytes, the scattered osmiophilic platelets gather gradually into an irregular, elongated heap lying along one side of the nucleus. It was my first belief, borne out by hematoxylin preparations, that this was the limosphere of authors, gradually forming by fusion of the platelets. It is clear now that the whole course of events is more complicated, and that the limosphere is by no means so simple a structure as has been supposed. Actually the osmiophilic platelets are thus assembled in connection with the formation of a new structure to which it will hereafter be convenient to limit the term "limosphere," regardless of the fact that as originally used by Wilson ('11) it probably involved the platelets proper to some extent at least. The limosphere appears first amid the platelets as an elongate body, often duplex in a later stage, but finally rounding up to form a conspicuous clear sphere within which is a large darkly staining granule (Figs. 11 to 14). This is the typical limosphere of Allen's account, its earlier stages having been overlooked for the most part by previous workers. During the later stages in its development, the osmiophilic platelets show a less intimate relation to the limosphere (compare Figs. 11 and 14), and subsequently the relation becomes very loose, the platelets being even more scattered and more independent of the limosphere or its remains. The limosphere now begins to elongate, its staining capacity (with hematoxylin or acid fuchsin) rapidly changes, and a portion of its substance is gradually separated off and deposited along the blepharoplast at the anterior end of the sperm (Figs. 17 and 18). The remnant of the limosphere (Fig. 18) takes no further part in the development of the sperm, although it undergoes further characteristic changes in morphology and staining capacity. The part deposited at the sperm tip is the so-called apical body. It begins very soon to elongate (Fig. 19), and by means of acid fuchsin I have been able to follow its later history and to show that it probably stretches out along the elongating nucleus, forming thus a rod-like "acrosome" comparable to that in some of the Pentatomidæ.

The interpretation of these phenomena seems fairly clear. The relation of the osmiophilic platelets to the developing limosphere suggests in every way the formation of the acrosomal material in those animals in which the Golgi bodies do not fuse to form a single, permanent mass—the acroblast. The structure of the limosphere is remarkably similar to that of many secretory granules and of the acrosomal vesicle and granule of animal sperms. The ultimate history of the apical body portion in the moss sperm is again exactly comparable to that of the acrosome in animals. The unexpected difference presented by the division of the limosphere does not, so far as I know, ever occur in animals. This unusual behavior together with the staining of the limosphere at this period (Figs. 17 and 18) led to my erroneous conclusion that the remnant of the limosphere was equivalent to the Golgi remnant in animals. It is clear, however, that the essential homologies which I first pointed out (Bowen, '26*b*) between the formation of the apical body and the osmiophilic platelets still hold. My conclusion that the osmiophilic platelets produce the acrosomal material in the moss sperm and that they are therefore equivalent to the animal Golgi apparatus can, therefore, now be reaffirmed. All the evidence available from sources of every kind bears out my contention that the osmiophilic platelets represent such part of the Golgi apparatus of plant cells as can now be certainly identified. It would be extra-ordinarily interesting to know whether the vacuole primordia, more particularly their enclosing membranes, play any joint rôle in the formation of the limosphere, and might thus be brought into line with the Golgi apparatus. But thus far there is not the slightest evidence of such a coöperation, indeed at present no vacuome has been demonstrated in the androcytes, and at the moment I am inclined to the opinion that no such coöperation occurs.

The particular mistake of my earlier account, which I wish now to make clear, has to do with an error in interpreting a stage like Fig. 14. It was my belief, in the absence of an extensive series of successful Golgi preparations, that the limosphere in early androcytes was an early stage in the history of the chondriome. The blackened granule and its enclosing vacuole gave to it an appearance exactly like that of the nebenkern in an insect sperm. I believed that the limosphere proper appeared only at a later stage. The whole picture (Fig. 14) is so strikingly like an insect spermatid that the error was not suspected by myself or by others who were familiar with osmic acid preparations. As a matter of fact, the moss androcyte does possess a remarkable similarity to a generalized animal spermatid, though not completely in the manner I suggested at first.

This clears the ground for a final disposition of the plastidome and pseudochondriome in the developing sperm. As regards the plastidome I have been as yet unable to follow its history, and its identification at any stage in the androcytes is still uncertain. It is possible that it degenerates, but it seems more probable that it either remains distinct and is disposed in some characteristic place in the sperm, or merges inextricably with the pseudochondriome. In the first case it would be practically necessary to consider the plastidome as an independent cytoplasmic component equal in rank with the chondriome and Golgi apparatus. In the second case the conclusion would seem justified that the plastidome is merely a specially differentiated portion of the pseudochondriome, as has long been contended by Guilliermond. Confusion between these two components in the spermatid led me to identify the plastidome with the pseudochondriome in my earlier report, and to draw the conclusion that the plastidome was therefore homologous with the animal chondriome. This inference now appears to have been based on inadequate evidence, and while it may be correct I prefer for the present to hold the whole matter of plastid homologies in abeyance pending my more extended study of moss material.

The pseudochondriome seems to occur in the earlier androcytes as a tangled mass of threads more or less encircling the nucleus and to some extent the developing limosphere. Eventually this material seems to spread out over the nucleus, and stretches out along the length of the sperm as the nucleus itself elongates. This general history and the ultimate arrangement of the pseudochondriome in the sperm lead me to believe that this material represents the chondriome of animal cells. That, indeed, the superficial similarities between an animal chondriosome and a plant pseudochondriosome are real, the pseudochondriome being in fact the chondriome of the plant cell, as Mottier ('18) for example has held, and also Guilliermond ('24) in the modified sense indicated above.

One further cytoplasmic component of a generalized nature remains to be considered—the central apparatus, central bodies, or centrioles, often called blepharoplasts by botanists. In general, plant cells are characterized by the absence of central bodies, and when something similar to them does occur, there has been much controversy as to whether such structures can properly be homologized with the central bodies of animal cells. Sperm-forming cells offer a remarkable opportunity for checking up on this matter, for the reason that the relation of the tail filament to the central apparatus in the sperm is beyond dispute. If in plant sperms a centriole-like body should be related to vibratile filaments, the conclusion that this body was a true centriole would appear unavoidable. According to Allen ('17) *Polytrichum* androcytes possess a small "blepharoplast" which eventually draws out into a rod-like structure applied to the elongated nucleus. The two free filaments characteristic of moss sperms appear to be inserted toward the anterior end of this rod, but Allen was able to follow their growth only in a fragmentary way. They seem suddenly to acquire a decided staining capacity in their completed condition. According to Wilson ('11) on the other hand, the moss androcytes possess a small blepharoplast which remains morphologically distinct from a filament (the bulk of Allen's blepharoplast) that grows from it posteriorly. My studies clearly support the latter description. At an early stage (Fig. 15) there is a small, definite blepharoplast or central body to which is attached a delicate filament. Subsequently (Fig. 16), the central body becomes somewhat larger and more conspicuous (as is common also in animal spermatids), and the filament grows rapidly back over the nucleus. When the nucleus begins to elongate, its anterior tip is attached to the posterior end of the centriole (Fig. 18), a relation which probably is maintained in the completed sperm. But the extraordinary thing is that the filament, at first apparently single, soon becomes clearly multiple. Benda preparations, which stain the filament very sharply, show at least two filaments which separate sufficiently at chance places to be clearly demonstrated. There are sometimes indications of a third filament, though this seems at present to be doubtful. It is my suggestion that these two filaments are the well-known free filaments of the mature sperm. After the sperm is well along in its differentiation they probably break loose from their association with the body of the sperm, and thus Allen's failure to follow them clearly in their growth would be logically accounted for. I believe that the observations here recorded leave no doubt that the body to which the filaments are attached is a true centriole comparable in every way to the central bodies of animal cells.

This brief outline of sperm formation in mosses clears up many points which long have been a puzzle to cytologists. It leaves the fate and possible homologies of the plastidome in an unsettled condition, but I have hopes that by the time my study is ended this gap will have been closed at least in part. The results here given in outline will be reported in detail in a series of papers of which the first will soon be ready for publication.

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EXPLANATION OF PLATE I.

Figures 1, 2, 4, 6 and 7 are from root-tips of *Vicia faba;* Fig. 3 from *Hyacinthus;* Fig. 5 from *Ricinus;* Fig. 9 is from *Polytrichum juniperinum;* Figs. 8, 10, 11 and 13 are from *P. commune;* Figs. 12 and 14 to 19 are from *P. piliferum.* Figs. 1 to 7 and 9 are reproduced at a magnification of approximately 1125 ×; Figs. 8 and 10 to 19 at 2475 ×.

FIG. 1. Osmiophilic platelets. Kolatchev.

FIG. 2. Plastidome in an early prophase. Benda.

FIG. 3. Osmiophilic platelets, plastidome and pseudochondriome. Kolatchev.

FIG. 4. Pseudochondriome. Champy-Kull.

FIG. 5. Vacuome. Weigl.

FIG. 6. Osmiophilic platelets, plastidome and vacuome. Kolatchev.

FIG. 7. Vacuome and osmiophilic platelets. Weigl.

FIG. 8. Early androgonia. Kolatchev.

FIG. 9. Tip of an antheridium with androgonia in a middle phase of multiplication. Weigl.

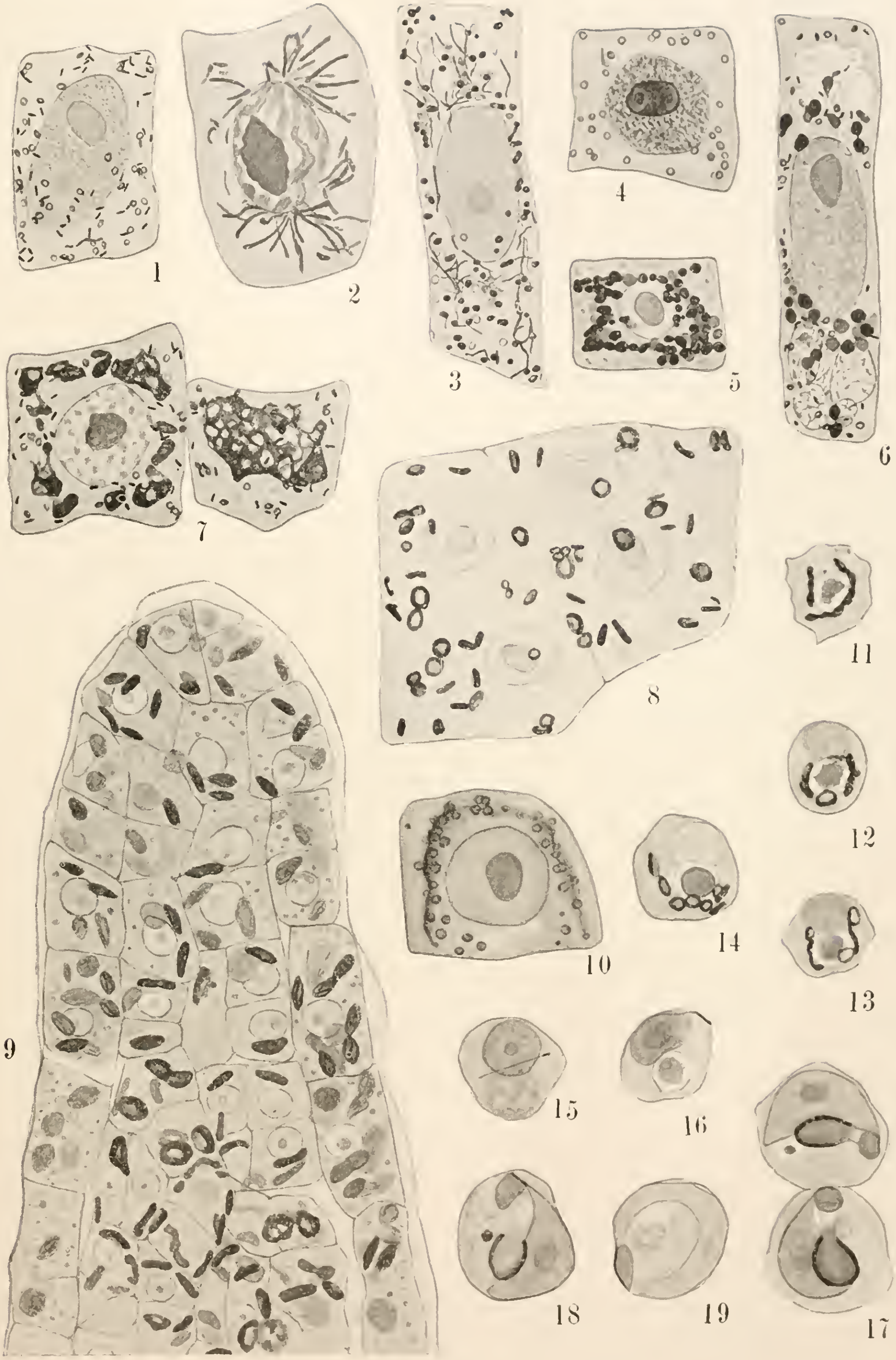
FIG. 10. Early androgonium. Champy-Fe-hematoxylin.

FIGS. 11 TO 14. Later phases of limosphere formation. Fig. 11 is viewed from one pole and does not show the nucleus. Kolatchev; Figs. 11 and 13 counterstained with acid fuchsin-aurantia.

FIGS. 15 AND 16. Centriole and tail filament in earlier sperm-forming stages. Benda.

FIGS. 17 AND 18. Stages in the deposition of the acrosomal material. The heavily stained periphery of the limosphere is now known to have little if anything to do with the osmiophilic platelets. Allen's fluid-Fe-hematoxylin.

FIG. 19. Early elongation of the acrosome. Benda.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R. H. BOWEN.

A PATHOGENIC LUMINESCENT BACTERIUM.

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In 1889 Giard and Billet (1) found that a number of different kinds of amphipod crustacea (*Talitrus*, *Orchestia*, *Ligia*, and others) gave off light. This luminosity was found to be caused by the presence of luminous bacteria in the tissues of the crustacea. In working with these luminous bacteria Giard and Billet were able to transfer them from one marine crustacean to another with ease and when grown on any marine crustacean tried, the bacteria became luminous. The authors could not get the bacteria to luminesce upon artificial media, although they obtained good growth. They offered no explanation for this failure and Harvey (2) pointed out that it seemed peculiar that these organisms could not be grown on artificial media so that they would produce light.

The luminous bacterium isolated by Giard and Billet is perhaps best known as *Bacterium Giardi* (Kruse) Mig., but much work is still needed upon the classification of luminous bacteria.

In August, 1925, several luminous individuals of *Orchestia platensis* (Kreyer) and *Talorchestia longicornus* (Say) were brought into the laboratory of E. N. Harvey at the Marine Biological Laboratory, Woods Hole, Massachusetts, and he turned them over to the author for investigation.

The sand flea chiefly studied was *Talorchestia longicornus* (Say) which can be found buried in the sand just above the high tide line during the daytime and feeding at the water's edge at night. This species is about the size of a honey bee and while they can be collected in June no luminous individuals have so far been found until August. These sand fleas will live several days in the laboratory if left in dishes of moist sand.

When luminous sand fleas were broken open and the grayish colored exudate mounted under the high power of a microscope, numerous small motile rod-shaped bacteria could be seen. Inoculation of some of this material from the sand flea upon sea water peptone agar to which had been added sufficient sodium hydroxide to bring the pH to 8.1 resulted in the appearance of numerous bluish-green luminous colonies of bacteria in from twenty-four to thirty-six hours. These colonies were picked and pure cultures obtained. By a series of transfers it was readily determined that this bacterium in pure culture growing upon artificial media of many different kinds would produce light if the proper osmotic pressure and hydrogen ion concentration were maintained. The optimum, minimum, and maximum salt concentrations and hydrogen ion concentrations for growth and light production have not been determined but sufficient evidence is at hand to show that growth and light production may take place in a wide concentration of salts and hydrogen ion concentrations, the greater range from sea water being toward fresh water and neutrality. Data on this phase of the subject will be published in another paper.

Thus it at once became evident that Giard and Billet were unable to obtain luminous organisms upon artificial media because they failed to adjust the hydrogen ion concentration of their media which became neutral to acid when they added peptones to sea water and in such a case good growth may be obtained but no luminescence.

Giard and Billet also did sufficient experimentation to satisfy themselves that this bacterium was pathogenic to sand fleas. They inoculated sand fleas and followed the cases through to the death of the organisms which they claimed occurred more quickly than normal sand fleas will die in the laboratory.

The author repeated some of this work and found that it was possible to transfer the bacteria quite easily from one crustacean to another and that the administration of large numbers of this bacterium to a sand flea did cause it to die sooner than normal fleas. There were cases, however, when transfer of bacteria or feeding bacteria seemed to have doubtful effect and the sand fleas failed to become luminous. This led the author to collect non-luminous sand fleas from various habitats and isolate bacteria from the intestinal tract. In almost every one of twenty separate sand fleas tried, luminous bacteria could be isolated and grew well and produced light on artificial media. The luminous bacteria isolated were quite surely *Bacterium Giardi*, but more proof is needed to demonstrate that this is the only species of luminous bacteria which inhabits the sand flea. This demonstrates, as pointed out by Dahlgren (3), that these bacteria are not necessarily luminous continuously, although it does not preclude the fact that they may be kept continually luminous without being passed through the sand flea, as has now been done for two years by the author. This observation also seems to point to the conclusion that this bacterium is a normal inhabitant of the body of the sand flea but that at certain times and under certain conditions it invades the muscles and increases in number so rapidly that the sand flea becomes luminous and dies. It is possible that the bacteria do not become luminous until they have spread to the muscular tissue of the sand flea and thus luminosity would mean that the host was doomed to die, since I have never observed a luminous sand flea recover. As to what causes the bacteria to invade the muscular tissue, especially in August at Woods Hole, Massachusetts, nothing yet is known. It may be a lowered resistance of the sand flea or increased reproductive activity of the bacterium due to a higher temperature or due to other causes. It should also be said that out of examination of possibly twenty thousand sand fleas only a very few ever become luminescent and it is quite possible that this bacterium plays a part in the death of thousands of sand fleas that never become luminescent. In other words, if this bacterium is really pathogenic, of which there is still some doubt, it is doubtful if there is any connection between luminosity and pathogenicity other than that great numbers of this bacterium under favorable environmental conditions naturally produce a maximum deleterious effect upon sand fleas and also give off more light.

SUMMARY.

Amphipod crustacea are the host of *Bacterium Giardi* which becomes luminous under certain conditions and may kill the sand flea.

This bacterium, if isolated in pure culture and grown upon peptone sea water agar of pH 8.1, becomes luminous within twenty-four hours and may be kept so by frequent transfer for at least two years.

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SOME FACTORS AFFECTING THE TOXICITY OF HYDROCYANIC ACID FOR INSECTS.

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During the last twenty years a vast amount of work has been done on the control of injurious insects by the use of hydrocyanic acid. Inasmuch as little quantitative work seems to have been done on the influence of environmental factors, such as temperature and humidity, upon the toxicity of HCN to insects, and since the relation of concentration of cyanide to length of exposure also does not seem to have been thoroughly investigated, it was thought advisable to study these points in some detail.

Apparatus and Method.

To determine the effect of concentration of cyanide, length of exposure, temperature and humidity upon the toxicity of HCN to insects, apparatus by which all these factors can be controlled or varied must be employed. In the present experiments, such apparatus was devised and proved to be very satisfactory.[2]

The apparatus, as shown in Fig. 1, consists of the following units: (*A*) flow regulator (one-inch glass tube 5 feet long); (*B*) humidifying unit, consisting of two half-liter bottles; (*C*) two flow meters; (*D*) generating flask; (*E*) half-liter bottle used as a fumigation chamber, placed in a water bath; (*F*) a small absorption bottle used for collecting samples of gas for analysis; (*G*) a one-liter bottle filled with NaOH for absorbing the gas. The outlet at H is attached to a suction pump.

The air flows in the direction indicated by the arrow. The rate of flow can be regulated by raising or lowering the glass tube

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[1] This work was done in coöperation with the American Cyanamid Sales Company.

[2] The writers wish to acknowledge the assistance given by Mr. K. D. Ashley, of the Chemical Staff of the American Cyanamid Sales Company, who furnished valuable suggestions for devising the apparatus and for the colloidal silver iodide method of analysis.

(*a*). The humidifying unit is filled with either distilled water or various strengths of sulfuric acid to give the desired humidity. The air, after passing through the flow regulator, passes over the humidifying unit and through the first flow meter, which gives the total rate of flow in liters per hour. The air line is divided after leaving flow meter No. 1; part of the air passes directly into the gassing chamber *E*, the remainder of the air goes through the second flow meter, thence to the generating flask *D*, which contains the source of HCN (calcium cyanide or liquid HCN). The air saturated with HCN mixes with the pure air in the tube leading to the gassing chamber *E*. The apparatus is so designed that the concentration of HCN can be regulated by controlling the amount of air which passes through the second flow meter. The reading of the second meter gives the rate of flow of air through the generating flask. The air-gas mixture, after leaving the gassing chamber, passes into the absorption bottle *G* containing a concentrated solution of NaOH for the purpose of absorbing the HCN, thus preventing any escape of the gas into the room.

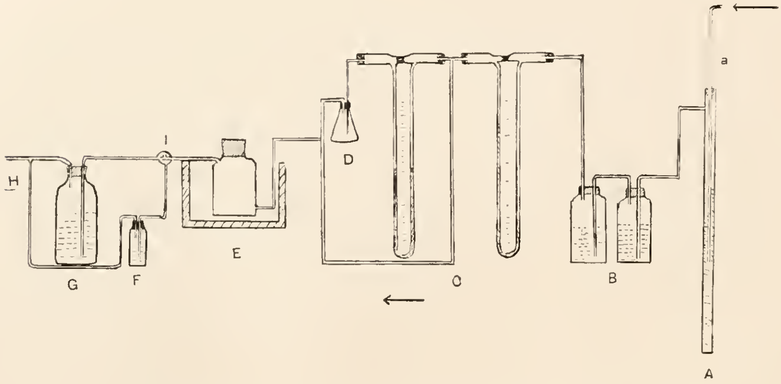

FIG. 1. Apparatus used in determining the toxicity of HCN to insects.

To collect a sample of gas for analysis, the gas-air mixture is passed through a small absorption bottle *F* by turning a two-way stopcock *I*. In this work two methods of analysis were used. The first method consists of collecting one liter of gas in a 10 per cent. NaOH solution and titrating with silver nitrate.

By the second method the HCN is passed into an alkaline, colloidal solution of silver iodide, which is made by adding potassium iodide to a known normality of silver nitrate. The time which is required to decolorize the solution is taken by means of a stopwatch. The first method of analysis, is perhaps, more accurate, but as it requires more time than the second, it was used only as a check on the AgI method. By the use of the second method, several samples could be tested during one exposure, so that accurate record could be kept of the concentration of HCN.

The insects used in the tests were kept in a container in a water bath maintained at a desired temperature for 1 hour before fumigating and the fumigation chamber was kept at the same temperature. After fumigating the insects, they were replaced in the water bath until the following morning, when they were removed and the mortality was determined. Throughout this work, unless otherwise stated, granular calcium cyanide was used as a source of HCN.

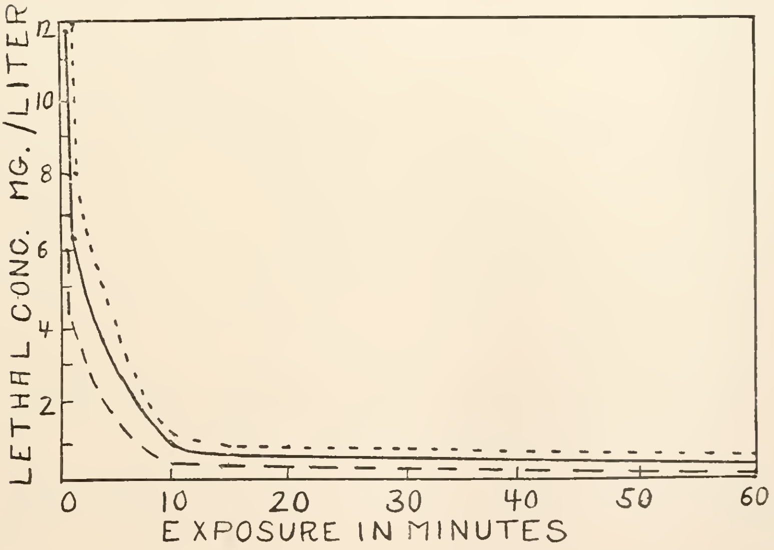

FIG. 2. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Macrosiponiella sanborni*. Solid line, 25° C.; dotted line, 20° C.; broken line, 30° C.

Relation of Concentration of HCN to the Length of Exposure.

Experiments were carried out to determine whether a low concentration of cyanide for a long period of time was more fatal than a high concentration for a short period of exposure. When using $Ca(CN)_2$ under field conditions, where one is not able to obtain a long period of exposure, it is necessary to use a very high concentration.

The results obtained from more than a thousand tests with two species of aphids and one species of thrips, show that concentration and length of exposure are inversely related up to a certain time, *i.e.*, toxicity = concentration × the time. Beyond a period of 15 minutes it seems that concentration is independent of the time, as it takes practically the same concentration to kill in one hour as in 30 minutes (Figs. 2, 3 and 4).

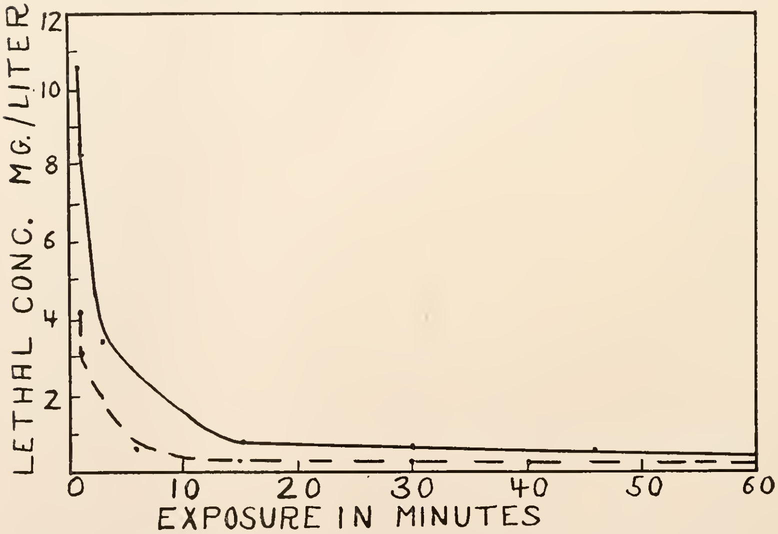

Fig. 3. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Aphis rumicis*. Solid line, 25° C.; broken line, 30° C.

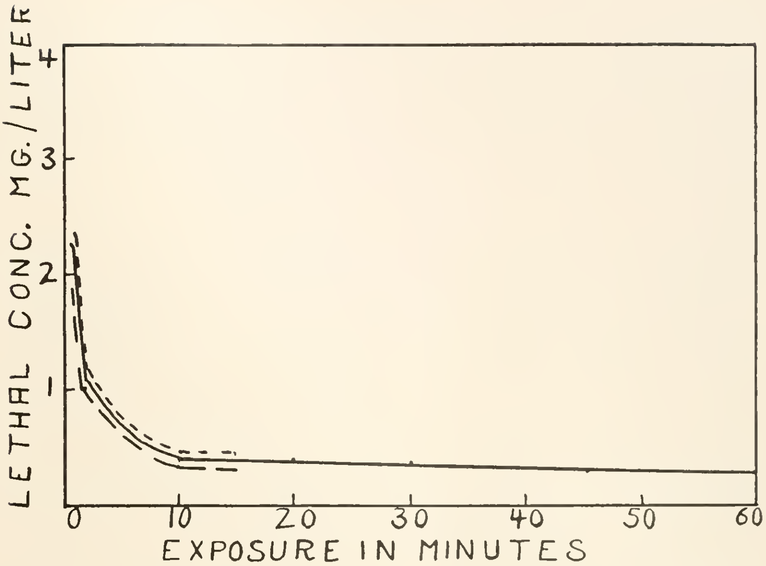

FIG. 4. Curve showing the relation of concentration of HCN to length of exposure and the effect of temperature upon the toxicity of HCN to *Thrips tabaci*. Solid line 25° C.; broken line, 30° C.; dotted line, 20° C.

TEMPERATURE.

It is known that metabolic processes increase with increase in temperature. With an increase in metabolic activities, we would expect an increase in the susceptibility of the insect to HCN. That such appears to be the case is shown in Figs. 2, 3 and 4. It may be noted from these figures that the effect of temperature is more pronounced with the shorter exposures.

Throughout the experiments on aphids, it was noted that the young organisms and the winged adults were more susceptible than the wingless adults. These results coincide with Child's[1] idea that the physiologically young organisms have a higher rate of metabolism than the older animals and are more susceptible to cyanide. In the case of thrips, however, the adults seem to be easier to kill than the young.

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[1] Child, "Senescence and Rejuvenescence," Chicago University Press, 1915.

HUMIDITY.

Several humidity experiments were conducted using *Aphis rumicis*, *Sitophilus granarius* and *S. oryza* under various relative humidities, other factors being the same. The result of these experiments indicate that humidity is not an important factor in determining the toxicity of HCN.

COMPARISON OF THE TOXICITY OF LIQUID HCN AND $Ca(CN)_2$.

During the course of the experiment, the question arose as to the comparative toxicity of hydrocyanic acid resulting from the action of water vapor on $Ca(CN)_2$ and the HCN volatilizing from liquid HCN. It is known that in addition to HCN, there are other compounds, such as ammonia and hydrogen sulphide, which arise from hydrolysis of $Ca(CN)_2$, and so it was thought advisable to ascertain what effect these foreign gases would have upon the toxicity of HCN.

The results indicate that the pure HCN coming from liquid HCN is slightly more toxic than the gas mixture which arises from $Ca(CN)_2$. These results are also confirmed in a number of experiments by adding slight quantities of NH_3 and H_2S to the gas which arises from liquid HCN, showing that there is some antagonistic action between the ammonia, hydrogen sulphide and HCN, thus lowering the toxicity of HCN.

EFFECT OF METHYL ACETATE ON THE TOXICITY OF HCN.

Some insects, such as *Melanoplus differentialis*, when placed in a sublethal concentration of HCN, close their spiracles and thus prevent, to a certain extent, the entrance of the cyanide gas.

It was thought that if some chemical could be mixed with the HCN, which would keep the spiracles open, the insects would be more easily killed by cyanide than if the spiracles are allowed to close. Methyl acetate is found to keep the spiracles open in *M. differentialis* when the insect is placed in an atmosphere of HCN. Experiments were conducted to determine what effect the addition of a small amount of methyl acetate would have upon the toxicity of pure HCN. The only insects available at the time of the experiments were *S. granarius* (grain beetle), and it was not determined whether or not the insects close their spiracles. However, when the methyl acetate was added to liquid HCN, the average kill as shown from ten experiments was 77.4 per cent. as compared with a 59 per cent. kill when pure HCN was used.

Summary.

A detailed study was made of the toxicity of HCN for certain insects. The insects used in this work were two species of aphids, *Aphis rumicis* and *Macrosiphoniella sanborni*, one species of thrips, *Thrips tabaci*, and two species of grain beetles, *Sitophilus granarius* and *S. oryza*. Apparatus by which all factors can be controlled or varied was employed.

The results of over a thousand experiments indicate that, within certain limits, concentration and length of exposure are inversely related or that toxicity = concentration × the time.

The higher the temperature, the more susceptible were the insects; this susceptibility was more pronounced with the shorter exposures.

Present indications are that humidity is not an important factor affecting the toxicity of HCN. Comparative studies on calcium cyanide and liquid cyanide show that the liquid HCN is more toxic than the gases from hydrolysis of calcium cyanide. A small amount of methyl acetate added to liquid HCN seemed to increase the toxicity of the gas arising from the liquid HCN, which may be due to the fact that a small amount of methyl acetate kept the spiracles open, while in pure HCN the spiracles were quickly closed.

THE NATURAL HISTORY OF *CUMINGIA TELLINOIDES.*

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WABASH COLLEGE (SEESSEL FELLOW 1926–27).

INTRODUCTION.

When it is considered that *Cumingia* is used extensively as a source of material for embryological and experimental studies it becomes a matter of importance to know its life history and its availability at all times. An observation made several years ago indicated that *Cumingia* exhibits a lunar periodicity in spawning and led the writer to make an extended study of the species covering a continuous period of five years. The laboratory work was carried on at the Marine Biological Laboratory at Woods Hole.

With the perfecting of the diatom method of feeding embryos it is possible to carry many marine larvæ through metamorphosis and thereby gain a knowledge of complete life histories where only fragmentary knowledge has been possible before. In this instance a pure culture of the diatom Nitschia, which was isolated at the Plymouth Laboratory, was used.[2] The diatoms at Woods Hole are not suitable for feeding because of the danger of bacterial infection of the embryos. The Beaufort species first used by Caswell Grave, on the other hand, grows in great abundance in aquarium jars and may be fed without difficulty. This diatom has the advantage over Nitschia in being relatively short and thick instead of long and slender. Both of these species should be propagated at marine laboratories for the use of investigators.

HABITAT.

Cumingia tellinoides is found principally in sandy bottoms containing a strong admixture of humus, with a sparse or moderate growth of eel grass, and where the tidal currents run swiftly as in narrow channels. The vertical distribution is not great, being a little above and below low tide mark.

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[1] From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn., and the Marine Biological Laboratory, Woods Hole, Massachusetts.

[2] The writer wishes to thank Dr. Isabella Gordon for a stock of this material.

The animal is readily dug with a spade at low tide and separated from the dirt with a coarse sieve. Under favorable conditions one can collect three or four dozen in the course of an hour. It has long siphons which enable it to lie inches below the surface of the bottom and yet feed from the water in the manner of lamellibranch mollusks generally, the food being chiefly diatoms and floating particles of organic matter. It is rather particular about the kind of bottom and the nature of the water currents and is therefore restricted in its distribution.

The Spawning Season.

Cumingia begins to spawn about the middle of June and continues until the middle of September. One can usually depend upon getting eggs in abundance until about August 20. At that time there is likely to be a lull or often a complete cessation in spawning until the first of September or until the next full moon. Eggs can usually be obtained in considerable quantity during the first half of September. Not all individuals are spawning in September and few are spawning heavily as a rule. Each adult spawns more than once during the season and the production of gametes is apparently continuous.

Method of Securing Eggs.

Cumingia spawn very readily in the laboratory but it is necessary to treat them according to schedule. Experience has shown that the best way to obtain clean eggs or sperm is to wash the animals free from all sediment and isolate them in small stender dishes half filled with sea water. When so placed in sea water and left undisturbed for an hour or less they extend their siphons and spawn if sexually mature. The eggs or sperm are thrown forcibly from the dorsal siphon as they accumulate in the supra-branchial chambers. The sexes are separate and may be distinguished by their color. The males are white and the females pink. The shell is not heavy so that the color of the gonads shows through more or less.

Cumingia cannot be kept in the laboratory indefinitely. It is desirable to use them the day they are collected, although it is sometimes possible to keep them for a day or two in moist sand. They must be kept away from water if it is desirable to have them retain their eggs or sperm.

The Egg. Size, Structure, Membranes.

The egg of *Cumingia* measures in extreme limits from .061 mm. to .065 mm. while the usual size is .062 or .063 mm. In color it varies from a light gray to a distinct pink. The animal pole of the egg is comparatively free from yolk and pigment so that it appears light in color. Centrifuged eggs show a beautiful banding due to the separation of materials of different specific gravity. These materials consist of yolk, pigment, oil and clear protoplasm.

The polarity of the egg is fairly rigidly fixed as shown by the fact that in centrifuged eggs the cleavage planes remain unchanged although the pigment and so-called formative materials may be driven to any part of the egg. This general problem was investigated by Morgan in 1910 and the phase of it referred to here was fully verified by myself in 1924. He was concerned with the influence of the "organ-forming substances" upon development.

Surrounding the egg there is a jelly membrane about .03 mm. in thickness or half the diameter of the egg. It is wholly invisible under the microscope but readily demonstrable by the use of a suspension of india ink in the water. One might suspect the presence of this jelly membrane by the circumstance that the eggs in a dish do not come into contact but are separated by a little space. This tertiary membrane has apparently been overlooked by investigators. There is also a distinct vitelline membrane which undergoes little or no visible change after fertilization. The membrane is not perceptibly lifted after the penetration of the spermatozoön so it may be proper to say that there is no fertilization membrane. This may account for the fact that the *Cumingia* egg is at the mercy of the sperm and polyspermy is common. At any rate it is desirable to learn whether this susceptibility to polyspermy is due to absence of one or more of the egg secretions that Lillie has shown to be concerned with the fertilization reaction in the Nereis egg, and which sweep supernumerary spermatozoa from the surface of the egg after the initiation of the fertilization reaction. It frequently happens that one can count ten or a dozen sperm nuclei within an egg that has been too heavily inseminated. Cases have been seen in which every egg showed polyspermy of this extreme character. When eggs are spawned the germinal vesicle has already broken down and the first polar spindle has formed. On this account the first polar body appears very promptly after insemination.

Morgan found that the egg of *Cumingia* is very easily injured by rough handling so that it may fail to develop normally beyond cleavage. At first he was unable to secure normal veliger larvæ from centrifuged eggs. He finally centrifuged the entire animal before it had spawned and, to his surprise, it spawned completely centrifuged eggs which developed normally, showing that the dislocation of the egg materials did not interfere with normal development. He came to the conclusion that the trouble he had experienced was due to injury to the membranes of the egg.

RATE OF GROWTH, METAMORPHOSIS AND LIFE HISTORY.

The polar body is of interest chiefly because of its great size and mode of formation. It seems to round up before it comes to the surface of the egg and to migrate out, breaking its way through the cytoplasm of the egg as the latter is pushed up into a rounded prominence. The vitelline membrane, being thick and elastic, is pushed up locally. As a curious anomaly, no doubt associated with polyspermy, one case was observed in which two polar bodies came off at the same time, side by side, and were followed after the usual interval by a third polar body. As a rule only two polar bodies are formed. The first one rarely divides, although its division has been observed now and then. In case of polyspermy the cell may go into the three-celled condition at the first cleavage, and the polyspermic embryos may be normal or abnormal. *Cumingia* would, in fact, be first class material for a study such as Boveri's on the Echinoderm egg.

The rate of development and age at metamorphosis are shown in Table I. The later stages were obtained by feeding the larvæ, by means of which they were kept alive for a month. One can distinguish the new parts of the shell, as added to the original larval shell, and so tell where new growth has occurred. It is quite likely that the development of the larvæ in the open ocean is more rapid and uniform than in the laboratory. However, the veligers seemed to thrive upon diatoms in a large aquarium jar kept agitated by a mechanical device, and in time a considerable number went through metamorphosis.

TABLE I.

SCHEDULE OF NORMAL DEVELOPMENT AT MEDIUM SUMMER TEMPERATURES, 20°–21° C.

First polar body	12 to 15 minutes
Second polar body	28 to 35 "
First cleavage	60 to 70 "
Second cleavage	90 to 102 "
Blastula	5 to 6 hours
Gastrula	7 to 10 "
Trochophore	12 to 18 "
Metamorphosing trochophore	20 to 30 "
Veliger complete	2 to 24 days
Metamorphosis of veliger into adult form	16 to 24 "
Sexually mature in one year	
Fully grown in four years	
Duration of life two to four years	

The measurements given in Table III. show that the veligers grew somewhat before metamorphosis but more rapidly afterwards. This is not fully in line with data on the rate of growth of *Chiton* (unpublished), which show that there is no measurable growth until after metamorphosis. *Chiton* seems to be unusual in this respect. The larvæ of *Cumingia* did not develop uniformly in this aquarium habitat. Some grew symmetrically, adding equally to the shell all around, and others grew asymmetrically. I cannot, therefore, be sure of the normal form. Moreover, there is very considerable variation in the proportions of the normal veligers of a single female. If variation is as extensive in all lamellibranchs as in *Cumingia* it is difficult to see how the early larvæ of different species can ever be distinguished satisfactorily by measurements, although Stafford has been able to recognize the older larvæ. The chief difference that I have observed between the young veligers of various lamellibranchs is in their general appearance. Some have a thicker shell than others and therefore refract light differently. As is well known the veligers of all lamellibranchs are so much alike that they can be distinguished, if at all, only by experts. The differences are largely indefinable and are apparent only after long study.

Table II.

Schedule of Development at Higher Summer Temperatures, 22°–23° C.

First polar body	7 to 9 minutes
Second polar body	27 to 30 "
First cleavage	45 to 50 "
Second cleavage	70 to 75 "

Eggs developing at the rate shown in Table II. may reach the complete veliger stage in from twenty-four to thirty hours. The rate of development which Morgan gives for *Cumingia* trochophores and veligers is considerably too slow, and his statement that unfertilized centrifuged eggs are intact after forty-eight hours is also erroneous. Unfertilized eggs fragment within twenty-four hours. It should be noted, however, that he was not particularly concerned with the rate of development and these oversights do not affect the validity of the conclusions he draws.

Table III.

Measurements at Various Ages.

Egg .061 to .065 mm.—average .062 to .063.
Blastula same as above.
Trochophore same as above but slightly elongated anteroposteriorly.
Two day veliger .074 x .090 x .055 mm. to .08 x .091 x .055 mm.
Ten day veliger .09 x .10 mm.
Two weeks veliger .095 x .105 mm.
Three weeks Cumingia metamorphosed .09 x .105 mm. to .097 x .155 mm.
Four to five months (size attained before first winter.), 6 x 5 mm. to 10 x 8 mm.
One year, 11 x 8 mm. to 13 x 9 mm. (sexually mature).
Two years, 14 x 11 mm. to 16 x 11 mm.
Three years, 17 x 12 mm. to 17 x 13 mm.
Four years, 18 x 12 mm. to 19.5 x 14 mm. (Largest specimens found).

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Experiments are in progress to learn the normal length of life of *Cumingia*. It appears that they are approximately half

RATE OF DEVELOPMENT AT VARIOUS TEMPERATURES.

Date.	Temp.	1st Polar Body.	2d Polar Body.	1st Cleavage.	2d Cleavage.
Aug. 20	18° C.	12–15 min.	40–45 min.	100–107 min.	150–160 min.
Aug. 20	18° C.	13–16 min.	50–55 min.	105–115 min.	154–165 min.
Aug. 21 (Two lots)	20° C.	*A* 13 min. *B* 12 min.	33 min. 32 min.	71 min. 68 min.	103 min. 101 min.
Aug. 20 (Two lots)	20° C.	*A* 10–13 min. *B* 11–13 min.	30–32 min. 31–33 min.	68–72 min. 69–73 min.	101–105 min. 102–105 min.
Aug. 18	20° C.	13–15 min.	33–35 min.	67–70 min.	100–105 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	73 min.
Aug. 10	22° C.	10 min.	33 min.	50 min.	75 min.
Aug. 10	22° C.	8 min.	28 min.	48 min.	72 min.
Aug. 18	29° C.	7–8 min.	20–22 min.	42–45 min.	62–67 min.
Aug. 21	30° C.	7–8 min.	19–20 min.	35–37 min.	54–57 min.
Aug. 28	33° C.	5 min.	20 min.	38–41 min.	58–61 min.
Aug. 20	35° C.	Tried three lots of eggs. None developed successfully. A few put off polar bodies and a few cleaved irregularly: one or two cleavages only.			

Note: These data show a rapid increase in rate of development from 18° up to 22° C. They do not show a proportionate increase in rate above that. 33° C. shows injury and irregularity. Eggs refused to cleave at 35° C.

It is only fair to say that this table should not be used for the calculation of temperature coefficients because the temperature was not kept rigidly uniform during the course of the experiments. It is sufficiently accurate for the purposes of this paper. It is possible to recognize end points to the minute although not all eggs cleave at the same instant as is indicated here.

grown and sexually mature in one year. I have the impression that they do not survive many years. Rings of growth on the shell show that they usually survive from two to four years, but the amount of growth after the third year is negligible.

In addition to the summarized data on the rate of development given in Tables I. and II. which are averages, I give the record of actual experiments in Table IV. It will be noted that the rates of development of several lots of eggs at temperatures ranging from 18° to 35° C. are given. It is interesting that the increase in rate of development from 18° to 23° C. is much greater than it is from 23° to 30° C.

The summer temperatures of the water at Woods Hole range from 18° to 22° C., the lower temperatures being those of June and late September, the higher being attained in July and August. The optimum temperature for the development of the *Cumingia* egg appears to be around 25° C. although the egg never experiences this temperature in nature.

The difference in rate of development of embryos between 18° and 25° is truly remarkable. One therefore does not speak of the normal rate of development of an embryo without considering the temperature. Because of certain interesting features of this table the writer expects later to describe more in detail the temperature coefficient of development of cleaving eggs.

POLYSPERMY.

Eggs spawned according to the method here described are convenient for study because they can be artificially fertilized at will. It is important to use only a drop of concentrated sperm suspension to fertilize a small stender dish of eggs, (in about 40 cc. of water) otherwise polyspermy results and abnormal development follows. As stated above, the *Cumingia* egg is particularly susceptible to polyspermy and it might be a legitimate problem for students of fertilization to undertake to explain why this is so, giving due consideration to Lillie's suggestion, *Jour. Exp. Zoöl.*, Vol. 14, 1913.

THE LONGEVITY OF THE GAMETES.

If *Cumingia* eggs are allowed to remain unfertilized they ultimately die and disintegrate, though it has been found by experiment that they vary greatly in their longevity. The eggs of some individuals are much more vigorous and survive for longer periods than those of others. The poorest lots of eggs fragment in from six to nine hours, and the best lots remain apparently normal from fifteen to twenty-two hours. The average lot of eggs begins to fragment at the end of nine or ten hours and 90 per cent. of them have fragmented after fifteen hours. We may say therefore that the average longevity of the unfertilized egg, during which normal fertilization is possible, is 10 to 12 hours. When using eggs older than these one may look for a considerable percentage of abnormal embryos. It is only the best lots of eggs which give normal development of all embryos when more than ten or twelve hours old although early cleavage may appear normal.

The average life of the sperm in the most dilute suspensions capable of giving 100 per cent. fertilization (1/6000 to 1/8000%) is 4 to 5 hours. The extremes are 3½ to 18 hours. If fresh eggs are added to these dilute sperm suspensions after nine or ten hours from forty to fifty per cent. of fertilizations often occur. On the average one can expect only 20 to 40 per cent. of fertilizations after nine hours and sometimes none at all. After ten hours they may fertilize from one to ten per cent. of the eggs. It was noted that abundant normal larvae developed from eggs that were fertilized by these aging spermatozoa up to nine or ten hours.

A sperm suspension of 1/500 to 1/750 % lives longer than the more dilute suspensions mentioned above. They may give 100% fertilization up to 9 or 10 hours and a considerable percentage of the spermatozoa may live for 12 hours. It was observed that they usually fertilize from one to five per cent. of the eggs from fifteen to twenty five hours. It is therefore apparent that the conditions existing in a concentrated sperm suspension are not the same as in more dilute suspensions.

The evidence goes to show that half of the spermatozoa that are shed into sea water under natural conditions die within five or six hours and that nine tenths of them die within ten hours. After a few hours most of the spermatozoa stop swimming and lie quietly except for a few lashings of the tail at intervals. In the presence of eggs they become greatly activated and again swim vigorously. A very few are capable of becoming so activated up to the time when the life of the suspension becomes extinct, and a few fertilizations result, (1 to 5 per cent.).

It is interesting to note that J. Nelson observed that oyster sperm in the presence of egg secretions would wear out their limited store of energy within an hour and become incapable of causing fertilization.

I have not observed that spermatozoa in very dilute suspensions quickly lose their power to fertilize eggs while still motile as described by Professor Lillie for *Nereis* sperm, but it was observed that in very dense suspensions spermatozoa retain their vitality and ability to fertilize from two to three days. This phenomenon has been observed also by Gemmil, Lillie and Cohen. Gemmil ascribes it to the nutritive quality of the spermatic fluid, while Lillie and Cohen show that "it is due, at least in part, to the inhibiting effect of CO_2 in solution or to hydrogen ion concentration." Of course this extreme condition is never met with in nature.

BEHAVIOR OF THE LARVA.

The larva becomes ciliated as a late blastula and, as an early gastrula it develops a strong prototroch and rises to the top. It swims vigorously until the trochophore fully transforms to the veliger. After becoming a typical veliger it soon settles to the bottom temporarily and from that time on it swims less and less frequently until it approaches the time of metamorphosis. Late veligers rarely swim and the velum appears to become more and more an accessory feeding organ. A certain amount of feeble movement is maintained until metamorphosis is completed, and the velum disappears. The velum persists until the form of the larva indicates that metamorphosis is practically complete though it becomes less and less vigorous after the first few days of active swimming.

LUNAR PERIODICITY.

As indicated by the foregoing statements *Cumingia* shows a lunar rhythm in spawning. The period of the first quarter is the period of restricted spawning and full moon until new moon or until nearly first quarter is the period of free spawning. During the summer of 1922 *Cumingia* was not spawning from August 15 until September 1. On September 1 spawning revived and eggs were obtained in abundance for several days following. (September 5 was full moon in 1922.) The cessation in mid-August may be described as sudden and the recovery in September was equally striking. This complete break was observed by others who were experimenting with *Cumingia* eggs. Records of other years show that such a clear-cut periodicity has been observed before but is not demonstrable every year. There is, however, almost without exception, a reduced spawning at the time of the first quarter of the moon not only in August but in June and July as well. There can be little doubt, therefore, that we are dealing here with a true lunar periodicity. There is always a period of from one to five days during the season when eggs are not obtainable after which spawning is revised.

SUMMARY.

The breeding season extends from the second week in June to the middle of September. The gametes are produced continuously and spawning occurs more than once. A rhythm is present which is interpreted to be a lunar periodicity.

The rate of embryonic development is variable but under usual conditions the larva metamorphoses in about three weeks. Growth during the first two years is comparatively rapid and average adult size is attained at the end of the second year. Fortunate individuals may continue to live and grow for four years. Sexual maturity is reached in one year and spawning is abundant although greater quantities of gametes are produced during the second year.

The egg of *Cumingia* is subject to polyspermy and abnormal development often occurs if too heavily inseminated. The unfertilized egg will retain its vitality for approximately nine to twelve hours during which it is capable of developing normally. The spermatozoa when set free in sea water usually die within four to nine hours, a few die within three hours and a few live for twelve or fifteen hours.

The *Cumingia* egg is favorable material for experimental study in that it is obtainable in abundance, is readily fertilizable artificially, and it withstands laboratory manipulation without injury.

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BIOLOGICAL BULLETIN

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THYROID AND GONAD AS FACTORS IN THE PRODUCTION OF PLUMAGE MELANINS IN THE DOMESTIC FOWL.[1]

BENJAMIN HORNING AND HARRY BEAL TORREY.

I.

In a recent paper, Zavadovsky (1925, 1) has described the production of white feathers on pigmented fowls following thyroid feeding. The dosage was excessive. Some of the birds succumbed; all were highly intoxicated. The survivors soon moulted. Back and wing feathers fell abundantly in the course of ten days, and were succeeded by feathers that were partly or wholly white. Flesh feeding did not produce these effects, nor adrenal gland. The author concluded that "the thyroid plays a specific rôle in the regulation of the growth and moulting of feathers and in their pigmentation."

In the next issue of the same publication, Zavadovsky (1925, 2) described an experiment in which a mongrel black hen under whose skin five dog thyroids had been grafted, developed tufts of white feathers at the seat of the graft. He also observed that "the new plumage which makes its appearance after the experimental moulting is apparently much softer than the old ordinary plumage."

Since 1921, we have had some hundreds of thyroid-fed fowls under observation, and have repeatedly noticed in their plumage occasional feathers with defects in pigmentation similar to those described by Zavadovsky. These have not been regarded as satisfactory evidence of a specific rôle of the thyroid in feather pigmentation. While they could be induced by thyroid feeding, this was especially true when the dosage was sufficiently large to induce structural defects in the vane as well. Similar defects could be produced by inanition alone. Furthermore, defective pigmentation appeared in birds that were not on a thyroid diet and showed no general signs of hyperthyroidism. When such defects in pigmentation appeared in birds receiving the dosage of thyroid commonly employed by us, namely, 1 gram of Armour and Company's desiccated thyroid to 5,000 grams of body weight, the non-pigmented areas, when they occurred at all, were usually limited to the tips of the feathers involved. This was strictly true for the feathers of the trunk. In wing quills, the extent of the defect was sometimes larger.

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[1] The work on which this paper is based was done in the Zoölogical Laboratory University of Oregon.

With these facts in mind, we are disposed to refer the striking change in plumage recorded by Zavadovsky to a metabolic disturbance induced by an excessive, essentially toxic dosage of thyroid rather than to a specific action of the latter on the pigment-forming mechanism. The results of the graft mentioned in his second paper are not unfavorable to this view. We have not seen the extraordinary casting of feathers in adult birds which we suspect of being a further sequel of toxic feeding. But the moulting process, like the pigment-forming mechanism, is subject to modification under the influence of non-toxic doses of thyroid. This has been referred to in a preliminary note (Horning and Torrey, 1923, 2) and considered more fully in a recent paper (1925, 1). In the latter, two kinds of results of thyroid feeding were discriminated, the one referable to nutritive (associated with toxic), the other to non-nutritive factors. And shortly after (1925, 2), changes in feather structure were described which were not referable to changes in nutrition or to intoxication.

In the present paper, another non-toxic, non-nutritive effect of thyroid feeding will be described, namely, an increased pigmentation. Whereas Zavadovsky's birds *blanched* and not infrequently died under the massive doses pressed upon them, our birds, with a daily ration of thyroid that permitted them to maintain their health, lay viable eggs and rear normal offspring, grew *darker*.

II.

This darkening of the plumage, to which we originally called attention in a preliminary note (1923, 1) was also observed by Cole and Reid (1924). The plate accompanying their paper illustrates very well certain typical color changes produced in the feathers of Brown Leghorns by thyroid feeding. With a view to repeating our observations (1922) on the appearance of plumage of the female type on young Rhode Island Red males as a result of thyroid feeding, these authors found in thyroid fed Brown Leghorn cockerels that had assumed adult plumage changes in feather form and structure that are probably typical responses of poultry in general, but which Crew and Huxley (1923), on a similar errand, failed to note.

What is true of Brown Leghorns in this connection is also true of Campines, Barred Plymouth Rocks and Rhode Island Reds. White Leghorns show similar changes in feather form and structure, though not in color. This exception is likely to prove the rule among other dominant whites as well. There is a definite correlation between structure and color in these experiments, however, which brings both pigmented and non-pigmented races into a single category.

While these different races responded thus similarly to thyroid feeding, they did not do so with equal intensity. Of our pigmented races, the Brown Leghorn was most affected, the Rhode Island Red least. These differences in reaction associated with differences of race, however, were not conspicuous, nor are they so interesting, as those associated with differences in sex.

The results of thyroid feeding are peculiarly conspicuous in Brown Leghorn males, especially in neck hackles, shoulders, backs and saddles, all feathers that are brilliantly colored in shades of red and show marked sexual dimorphism. In the male, these feathers are distinguished by a lacy border of naked barbs (Fig. 1). On a thyroid diet, the latter tend to clothe themselves to their tips with extensions of the normal two rows of barbules (Fig. 2). As the barbules thus extend toward the edge of the feather, so also does the melanin pigment; for, as a rule, this pigment is carried by the barbules and limited by their distribution. As a result of thyroid feeding, the zone of barbules

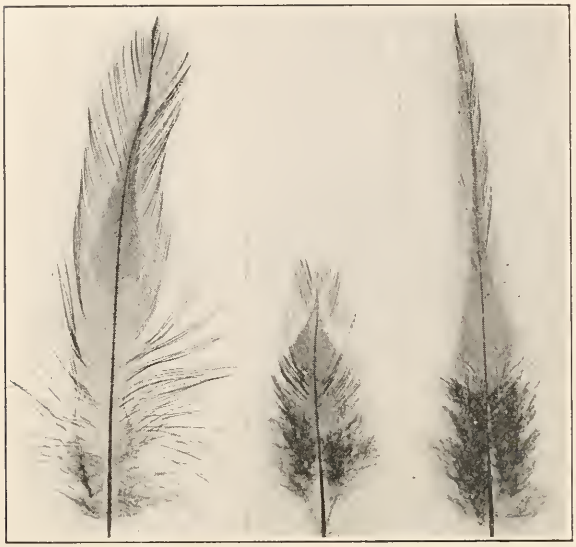

FIG. 1. Representative feathers from a normal adult B. L. male. From left to right they are: hackle, shoulder, saddle.

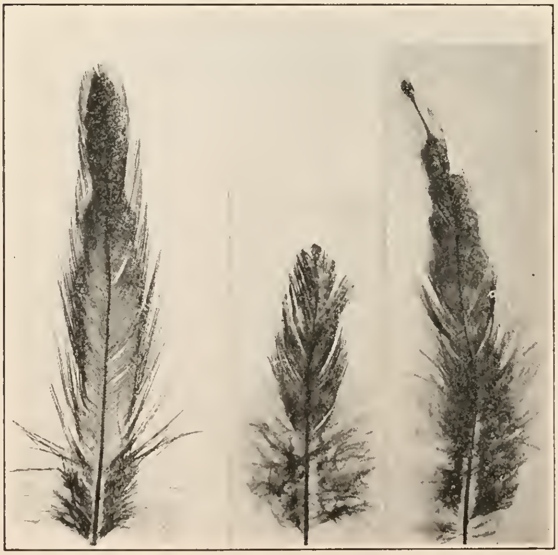

FIG. 2. Representative feathers from a thyroid fed adult B. L. male. From left to right they are: hackle, shoulder, saddle.

may extend beyond the zone of melanin pigmentation; but the reverse does not occur. In control birds, the reverse *does* occur. This is especially clear in the shoulder feathers of Barred Plymouth Rocks, in which dark bands pass distally across the naked barbs (Fig. 3).

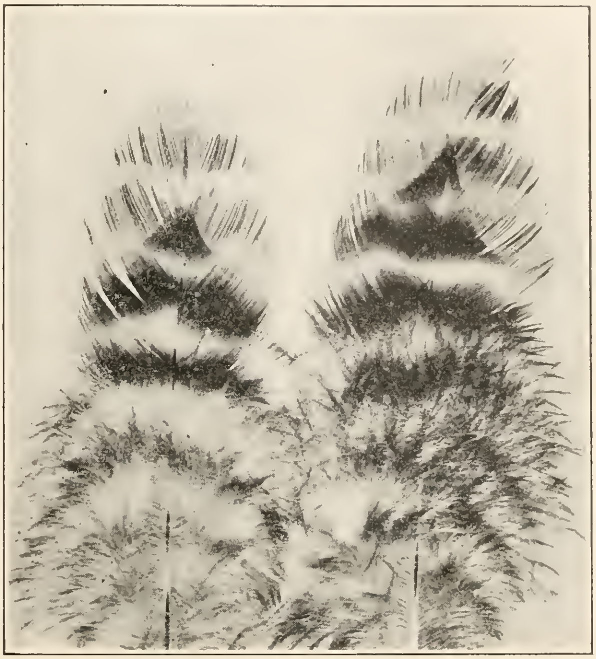

FIG. 3. Representative shoulder feathers from an adult Barred P. R. male, showing barring across naked barbs.

This tendency of barbules and melanin pigmentation to extend together over the feathers of thyroid fed birds is especially marked by reason of the fact that the zone of barbules in male birds possesses a typically notched or wavy contour with which the outer edge of melanin pigment distribution perfectly coincides. It is difficult to avoid the conclusion that the effective stimuli for barbule and for melanin formation in these feathers are one and the same. There is reason, however, for believing that this is not an adequate summary of the situation.

Everything that has been said about the male bird applies as well to the capon. In the latter, such decorative feathers as the hackles, saddles and sickles are longer and possibly more highly colored. The plumage is more luxuriant, *more masculine.* That it becomes more deeply pigmented with melanins as a result of thyroid feeding, however, does not appear from our observations.

In sharp contrast with the effect of thyroid feeding on the coloring of the male bird is its effect on the normal female. Our evidence on this point has been obtained first, from females that had received a daily ration of thyroid approximately 1 : 5000 body weight for at least a year, beginning with the second to fourth week after hatching; second, from females approximately two years old that had been plucked in hackle and saddle regions to stimulate the eruption of new feathers and then placed on the same ration of thyroid as the others.

In the first group, thyroid feeding produced no departure in coloration from the controls. One conspicuous instance of this absence of thyroid effect was supplied by three hens whose parents as well as themselves had been on a thyroid ration practically all their lives. When a year old, they were not distinguishable from their controls.

In the second group were five hens. In the new feathers that appeared on the denuded areas during the period of thyroid feeding there was little or none of the brown mottling characteristic of the controls, and a correspondingly wider distribution of dark pigmentation, less intense than in corresponding feathers of thyroid males, but present in all the experimental birds.

If these observations accurately represent typical conditions, it may be said that the plumage of Brown Leghorn females darkens under the influence of thyroid feeding, as indicated, but not to the same degree as in males of the same age, and apparently not at all in birds in their first year.

A comparable difference in the effect of thyroid feeding on the two sexes has also been observed among Barred Plymouth Rocks. It is the male, rather than the female, whose plumage darkens in the course of the early moults.

These facts suggest a possible inhibitory influence of the ovary in this connection, a view which receives strong support from the following case.

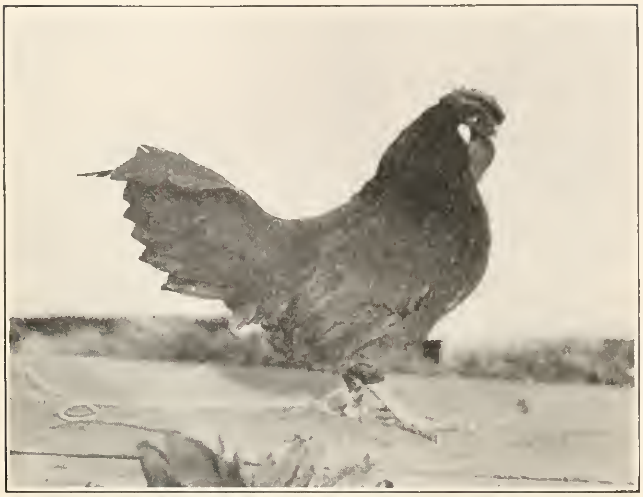

FIG. 4. Thyroid fed B. L. female from which the ovaries have been removed.

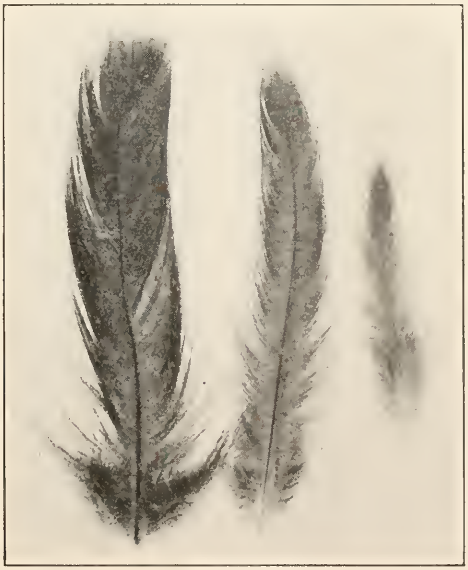

FIG. 5. Representative hackles from the bird shown in Fig. 4, arranged from below upward.

A Rose Comb Brown Leghorn female, hatched April 9, 1923, was ovariotomized May 6, 1923. Thyroid feeding was begun at once. Fig. 4 is a photograph of this bird almost two years later, just before she was killed on March 28, 1925. The comb and wattles are large and well formed. The spurs are 2 cm. long. Sickles are present among the feathers of the tail. These are all male characters, assumed as a consequence of ovariotomy. The body conformation is of the female type, as is, in general, the plumage. One exception to this statement has already been noted, namely, the presence of tail sickles. Another is to be found in the neck hackles which, though not well shown in the photograph, approximate in form and structure and color the hackles of thyroid fed male birds. In general, the plumage is duskier than in unaltered females. This is especially true for hackles and saddles. The former may have no lacing, or they may be laced intermittently in the fashion characteristic of thyroid fed males. In neither case, however, do traces of the mottling remain that is so conspicuous in the female hackle. The saddles, which are female in form and structure, show traces of mottling but along with the hackles show an intensity of melanin pigmentation that surpasses the results, mentioned above, on unaltered thyroid fed females.

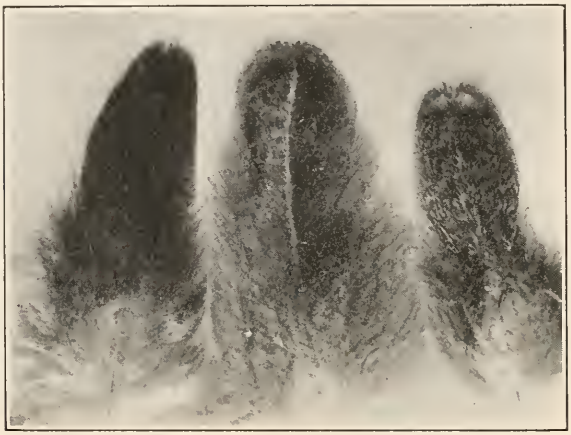

Fig. 6. Representative saddle feathers from three adult B. L. females which are, from left to right; the bird shown in Fig. 4, a normal thyroid fed female, and a control.

In post mortem examination of this bird, no trace of a definitive ovary was seen. At the site of the latter, however, were certain small fleshy nodules which revealed, on sectioning, masses of tubules resembling testicular tubules in various stages of development from sex cords, though lacking in definitive sex cells. We have assumed no connection between this tissue and the changes in pigmentation before us. Its significance appears to lie in another direction; and its further consideration will be deferred to another occasion.

While barbule formation and melanin production are both stimulated by thyroid feeding in the male, the presence of the ovary in the thyroid fed female interferes with the second without interfering with the first. Indeed, barbule formation reaches its fullest expression in the female. Lacing, though present in the hackles of the female to a limited extent, is an elaborate and striking feature of those feathers in the male that are especially associated with sex.

The evidence is obviously not sufficient to warrant final conclusions regarding the inhibitory effect of the ovary on pigment formation stimulated by thyroid feeding. It is clear, however, that males and females respond differently to thyroid feeding. And that this difference is due in some measure to the influence of the ovary seems equally true. As for the measure of its influence and the nature of the mechanism by which it is achieved, the matter is not so simple and must await further investigation.

III.

Two classes of feather pigments have been distinguished: *lipochromes*, which are soluble in alcohol and ether and are red, rose brown, yellow and scarlet, and *melanins*, which range from sepia to black. In our experiments, the extension of the latter is correlated with the disappearance of the former. Cole and Reid mention "an evident action toward the reduction of red pigment." We have no evidence of an actual replacement of the one by the other, either by substitution or transformation. It is quite consistent with our observations to assume for the present that the former are merely concealed as the latter advance.

The melanins appear in definite chromatophores, being formed *in situ*. The chromatophores are found in the epidermis of the rachis and barbs. At first they are lacking in pigment which is gradually laid down within them in the form of granules. The pigment of the barbule cells is obtained by a direct transfer from the chromatophores which send out amœboid processes as the barbules develop, these processes meeting and fusing with the barbule cells as though guided by a tropistic factor. Readers who are interested in the details of this remarkable mechanism are referred to the papers of Strong (1902) and Lloyd Jones (1915).

The expansion of melanins in the feathers of thyroid fed birds is conceivably dependent, then, on several factors: an increased amount of melanin pigment; an expansion of individual melanophores, with or without added pigment; an increase in number of melanophores; a migration of melanophores. Considering the large areas ordinarily free of melanins that darken under thyroid feeding, it is difficult to avoid the conclusion that the total amount of melanin is increased. The extension of individual melanophores over very much smaller areas is certainly a fact. Whether or not the melanophores multiply we have not determined. The number of *functional* melanophores assuredly increases. We have no evidence of the migration of melanophores over the long distances required by the observed facts, and it appears to be highly improbable.

The darkening of feathers as a result of thyroid feeding is probably due chiefly, then, to an increase in melanin pigment and an increase in the number of melanophores functioning with or without an increase in the number of cells themselves. Thyroid feeding is thus conceived to promote pigment formation, directly or indirectly, and possibly cell division as well. These are two sufficiently diverse functions, both, in our opinion, indirect. Whether the melanophores do or do not multiply under thyroid influence, there is no doubt that other cells do, namely, the cells of the barbules whose number and extent is so markedly increased. But it is not clear at present in either case through what channels the thyroid acts to augment pigmentation or cell proliferation. Evidence has been given elsewhere (Torrey, Riddle and Brodie, '25) for the view that thyroxin depresses the division rate when acting directly on Paramecium.

Summary.

The results of thyroid feeding on five breeds of domestic fowl are recorded. They vary with dosage and with sex. When the daily ration is compatible with good health, the males of pigmented breeds, both normal and castrated, tend to *darken* conspicuously owing chiefly to an increased production of plumage melanins. Normal females are affected, if at all, to a much slighter degree. Castrated females, however, resemble males in this respect as in others. *An antagonism thus appears between ovary and thyroid* as indicated by their relation to the formation of plumage melanins. The *blanching* of plumage observed after excessive doses of thyroid is attributed to a toxic effect, non-specific in character. A correlation between the effect of thyroid feeding on melanin production and feather structure is pointed out. White Leghorns show the latter but not the former, being dominant whites.

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THE pH OF THE BLASTOCŒLE OF ECHINODERM EMBRYOS.

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The development of the calcareous skeleton in Echinoderm embryos has been correlated recently with changes in the hydrogen ion concentration (1, 2, 3). This work was based on the results of Rapkine and Prenant (1) who injected indicator dyes into the blastocœle at the various stages of the developing embryo. They found that the pH of the blastocœlic fluid is 7.3 in the blastula, 8.5 in the early gastrula when the mesenchyme and the calcareous spicules appear, and 7.3 in the pluteus.

More recently (4, 5) it has been definitely shown that an evolution of acid occurs when protoplasm is punctured or torn. In view of this, the experiments for determining colorimetrically the pH of the blastocœlic fluid were repeated on Echinoderm larvæ obtainable at Woods Hole.[1]

The indicator dyes used were those of Clark and Lubs (6) and ortho cresol phthalein. They were prepared with NaOH in .4 per cent. aqueous solutions. All the solutions except that of brom thymol blue when injected proved to be quite innocuous to the embryos.

These dyes in sea water, obtained from the laboratory tank, give a color which indicates a pH of approximately 8.4 uncorrected for salt error and for the possible loss of CO_2 in the hanging drops in which the determinations were made.

I. Injection Experiments.

Asterias.—The early blastomeres of the *Asterias* egg are so weakly adherent that they would fall apart except for the

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[1] We wish to take this opportunity of expressing our appreciation to Dr. Isabella Gordon of the Imperial College of Science, London, for supplying us with late stages of Echinarachnius and Arbacia larvae from cultures which she was growing at Woods Hole during this past summer.

enclosing fertilization membrane. It is not until the eggs reach the 32, 64 or even 128 celled stage that the blastomeres become sufficiently adherent to form a comparatively intact wall about the segmentation cavity. Dyes, injected before the cavity becomes completely enclosed, immediately escape through the gaps between the blastomeres. In later stages the injected dye is retained for a longer period. The color of the dye, however, always begins to fade within a minute or two after the injection and in no case could an appreciable color be detected after 5 to 10 minutes.

The stages injected included the coarse and fine celled blastulæ, early and late gastrulæ, and Auriculariæ of five and ten days growth. In every case the colors of the indicators within the blastocœle were identical with those of the indicators in the environing sea water.

Echinarachnius.—The early blastomeres of the *Echinarachnius* ovum are more adherent than those of the *Asterias.* In the two and four celled stage the hyaline plasma layer is strong enough to press the two blastomeres closely together (7). By inserting micropipette through this layer and between the contiguous blastomeres the injection of the dye solution creates a temporary blastocœle. The blastocœle of early and late blastulæ, gastrulæ and plutei were injected with the dyes. In every case the colors assumed by the dyes were those typical for the pH of the surrounding sea water.

Dyes were also injected into the blastocœle of a pluteus fifteen days old containing a young echinus-rudiment. The dyes diffused through the blastocœle and into its prolongations in the lips and arms of the pluteus. The color of the dye in the blastocœle was always that of the dye in sea water and always faded within a few minutes.

In order to test the effect of changing the pH of the external environment blastulæ, gastrulæ, and early and late plutei, placed in a hanging drop of normal sea water, were injected with dye solutions and were then transferred into acidified sea water the pH of which was still consistent with viable conditions (pH 6.6 ±). The color in the blastocœles changed within a few seconds after immersion to correspond with the new pH. On being returned to normal sea water, the color quickly changed to that characteristic for the external medium. This procedure could be repeated a number of times until there was not enough appreciable color left within the blastocœle of the living embryos.

Blastulæ were also immersed in sea water whose pH had been brought to 5.4 ± and to 6.0 ±. Three minutes later they were injected with chlor phenol red and brom cresol purple. In every case the color within the blastocœle was the same as that of the dye in the surrounding medium.

Arbacia.—In the *Arbacia* egg the hyaline plasma layer is more prominently developed and much stiffer than in either the *Asterias* or *Echinarachnius* egg. Consequently, the blastomeres are always closely pressed together. The blastocœle appears early as an enclosed cavity; it does not enlarge to the same extent as in the other forms and its cellular wall, during the blastula stage, is much thicker. Therefore, when a pipette is introduced into the blastula a considerable amount of injury usually occurs at the spot where the wall is punctured. The disintegrating material of the injured cells is carried into the blastocœle and the consequent addition of acidified material, which has a pH of 5.4 to 5.6 (5), is sufficient to lower the pH of the internal fluid to that observed by Rapkine and Prenant, viz., 7.0 to 7.3. The color of the dye disappears within several minutes, the time varying with the dye used and the amount injected. This acid reaction in the *Arbacia* at this stage is so readily produced that it was not suspected as an injury phenomenon until experiments were performed on the larger and less readily injured blastulæ of the *Asterias* and *Echinarachnius*. Extra precautions were, therefore, taken to avoid injury and micropipettes were used the tips of which taper rapidly into very slender and elongated shafts. With such a pipette it was found possible to perform the injection with no sign of injury by causing the tip of the shaft to pass between and not through the cells of the blastula at the spot of puncture. The observed pH was then always that of the surrounding sea water.

When gastrulation begins the aboral end of the embryo dilates and the wall of the blastocœle becomes so thin that a puncture produces no appreciable injury. Consequently no special precautions were necessary and the injections all indicated the same pH as that of the external medium.

Later, when the pluteus stage is reached the archenteron occupies a considerable portion of the blastocœle. When, therefore, the pipette is inserted one is liable to injure the surface of the archenteron. The resulting injury to the archenteron immediately produces sufficient injury to affect the pH of the fluid in the blastocœle. By avoiding injury it was found that the pH of the blastocœle in the various stages of development of the *Arbacia* until metamorphosis is identical with that of the surrounding sea water.

The extreme susceptibility to injury of the cells in the wall of the archenteron is a striking phenomenon. If a pluteus is injected with phenol red and the surface of its archenteron slightly scratched a flash of yellow spreads over the archenteric wall, quickly followed by a return to the red color of the dye in the blastocœle. The cells, themselves, remain colorless and apparently normal. The irritation at the spot of injury seemed to spread from cell to cell and to cause them to produce an acid. This resembles the production of an evanescent acid zone around a mechanically injured starfish egg (5).

The archenteric cavity of a late pluteus was also injected by inserting the micropipette into the blastopore, and, in the few tests performed, the pH recorded was the same as that of the surrounding sea water. If too much pressure is applied the blind end of the archenteron breaks and the injected fluid flows into the blastocœle.

The blastocœle of the so-called bottom swimmers were also injected. These are sluggishly moving blastulæ containing disintegrated material from dying cells which are squeezed out of the blastular wall. The smaller size of these abnormal blastulæ and the accumulated debris in their interior probably account for their inability to acquire the pelagic habits of the normal forms. The injection of dyes into the blastocœle of bottom swimmers indicate a pH below 7.0. As long as disintegration is a continuous process the acid reaction of the blastocœle fluid is maintained. When, however, disintegration ceases and the blastula begins to recover, the pH of the blastocœle gradually rises until it reaches that of the surrounding sea water.

II. IMMERSION EXPERIMENTS.

Embryos in all stages of the starfish, sanddollar, and sea urchin were immersed in bowls of normal sea water deeply colored with brom cresol purple, phenol red, meta cresol purple and cresol red. After an immersion of ten minutes to half an hour the embryos were removed, rapidly washed, and placed in fresh sea water. In every case, the dye penetrated the blastocœle and indicated a pH of the seawater. These colored embryos were then placed in sea water having a pH of 6.6 ± (sea water acidified with KH_2PO_4) and the color changed, within a few seconds, to that characteristic of the environing sea water.

III. CONCLUSION.

The microinjection of dye indicators (see Table), and the immersion of embryos in sea water colored with dye indicators show that the fluid in the blastocœle of the normally developing embryos of *Asterias forbesii*, *Echinarachnius parma*, and *Arbacia punctulata* has the same pH as that of the environing sea water. This is true for all the stages of the embryos from the time that the blastocœle first appears until metamorphosis.

Indicators.	Blastocœle.	Sea water.	pH.
Phenol red	Red	Red	> 7.6
Cresol red	Red	Red	> 8.0
Meta cresol purple	Red wine colored	Red wine colored	> 8.2
Thymol blue	Yellowish green	Yellowish green	< 8.6
Cresol phthalein	Colorless	Colorless	< 8.6

The fact that the color of the dye in the blastocœle always changes within a few seconds to that typical for the pH of the environing medium whether acid or alkaline, and the fact that the color disappears from the blastocœle of embryos in sea water in which no dye was present, indicate that the wall of the blastocœle of a normal embryo is freely permeable.

The variations in the blastocœlic pH recorded by previous investigators can be accounted for by the ease with which acid is evolved in the sea urchin embryo when the cells of the walls of the blastula and the archenteron of the pluteus are injured.

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THE PERMEABILITY OF FROG SKIN TO UREA.

I. THE INFLUENCE OF NaCl AND $CaCl_2$.

M. L. BASKERVILL.[1]

Although there is already an extensive literature relating to cell permeability, many more facts are needed before an adequate explanation of the mechanism involved can be expected. So much of the work reported has been of a qualitative or even of an indirect nature that additional data are needed on the quantitative side, and especially with substances occurring under normal physiological conditions. Accordingly a quantitative study of the penetration of urea through living frog skin under the influence of various reagents has been undertaken. In this paper are reported the effects on the process of different proportions of NaCl and $CaCl_2$. Experiments with other reagents will be reported in subsequent papers.

For a number of reasons urea is an excellent substance for use in studying permeability. It is an organic compound occurring normally in animal tissues and fluids and as such belongs to a class needing much more study. It penetrates tissues with moderate ease, entering neither too quickly nor too slowly for experimental purposes. A sufficient amount of urea for quantitative determination passes through the tissues within a period of time that insures the skin's remaining alive and in healthy condition. This point is of much importance because there is reason to believe that some of the papers on the permeability of frog skin have reported experiments extending over such a length of time that the skins were killed during their progress. On the other hand, the penetration of urea is not so rapid that the amount may not be decreased by agents having an inhibiting effect on permeability. Of practical importance is the fact that direct quantitative determinations may be made so readily on urea.

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[1] From the Laboratory of Physiology of the University of Pennsylvania; the Marine Biological Laboratory, Woods Hole; and the School of Medicine, University of Texas, Galveston.

There has been little work reported on the effect of NaCl and $CaCl_2$ on cell permeability to non-electrolytes. The number of papers relating to the effect of these salts on permeability to other electrolytes, or concerned with their effects in general on cell permeability, have been so many that only a few typical ones to illustrate the different kinds of studies may be mentioned here. Osterhout (1) showed that there is a difference of conductivity with different proportions of NaCl and $CaCl_2$ in the case of various plants, and he proved that this also holds true for frog skin (2). However, in a more recent paper (3) he has expressed the view that an increase of conductivity does not necessarily indicate a greater permeability to ions in the absence of electric currents. Harvey (4) noted that NaOH enters Spirogyra cells more easily from a NaCl solution than from a mixture of NaCl and $CaCl_2$. R. Hamburger (5) found that artificial edema may be produced by solutions lacking calcium. In 1909 R. S. Lillie (6) reported that pure isotonic solutions of sodium salts increase the permeability of the pigment-containing body cells of Arenicola larvæ, while this effect is prevented by addition of $CaCl_2$. Many other papers by the same author refer to similar effects of these salts in relation to permeability. In a paper dealing with artificial parthenogenesis, Lillie and Baskervill (7) assumed that the greater effect of NaCl as compared with mixtures of NaCl and $CaCl_2$ in causing development is due to the increase of permeability in the former case. Since it has been so often assumed that NaCl will increase the permeability of tissues for all substances and that certain proportions of $CaCl_2$ will decrease permeability, it seemed desirable to ascertain how much the salts in question might influence the permeability of frog skin for a non-electrolyte, urea.

The skin used was obtained either from the bull frog, *Rana catesbiana*, or from *Rana virescens*, and was removed with as little injury as possible. Usually it was tied over the end of a glass tube 16 mm. in diameter. In a few experiments the skin from a whole leg tied at the distal end to form a sac was used. Measured amounts of the salt solutions under investigation were put into the tube or sac. The skin was then immersed in a bottle containing 50 to 100 cc. of urea solution, an amount large enough to prevent any significant change of concentration during the experiment.

The skins were used in symmetrical pairs; so that with each pair of skins taken from corresponding parts of the two sides of the frog, one was treated with NaCl (0.7 per cent.), and the other with isotonic $CaCl_2$ (1.1 per cent.), used either alone or in combination with the NaCl solutions. All references to salts in this paper are to solutions of the above mentioned concentrations.

After a convenient interval of time, varying usually from two to seven hours, the amount of urea that had penetrated the skin was measured by the Van Slyke-Cullen method (8). Numerous experiments were performed because conclusive results could be obtained only by frequent repetition of each experiment in order to compensate for the high degree of variability in the skin, in part seasonal, in part individual. With frogs that had been in captivity for some months, and especially with frogs that had developed "red-leg," no significant difference in the permeability could be obtained. However, even in these skins such slight differences as were noted were virtually always in the same direction as in normal skin.

In most of the experiments a 5 per cent. solution of urea was used, as lower concentrations did not yield significant differences. In order to subject, as far as possible, all of the cells of the skin to the desired proportions of salts, the urea was usually dissolved in the same isotonic salt mixtures as were used on the other side of the skin. Although the objection might be raised that this strongly hypertonic solution penetrates readily by reason of injuring the tissues, there is evidence to the contrary. Skins that have been killed allow equal quantities of urea to pass whether NaCl or NaCl and $CaCl_2$ is used; the fresh skins, however, show marked differences of behavior under the conditions of the experiments. Furthermore, if an isotonic solution of urea, *i.e.*, 1.5 per cent., is used with one of each pair of skins in a series, and the other skin is treated with 1.5 per cent. urea solution brought up by means of glucose to the same osmotic pressure as a 5 per cent. urea solution, there is no essential difference in the passage of urea in the two series. For these reasons it is felt that the experiments reported are no more open to objection than any experimental case where solutions other than lymph and water are used on the inner and outer sides, respectively, of the frog skin.

Following are the results from several typical experiments. When the inner side of the skin is against the tube and so in contact with the pure salt solution, while the outer surface of the skin is in contact with urea, the direction is designated as "normal" and the reverse direction as "turned."

The data in Table I. show the kind of results obtained when skins from whole legs were used. The skins were turned in the "normal" direction and each contained 5 cc. of the solution indicated. They were washed in the respective solutions for $1\frac{1}{4}$ hours before the experiment started. Three per cent. urea dissolved in the same salt solution as used on the inside of the skin was employed in each case. Penetration was allowed to continue three hours.

TABLE I.

Date of experiment 10/26/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	34.02	35.88
10	90	16.26	30.60
15	85	15.66	33.36
25	75	23.82	28.02
50	50	25.14	30.30
100	0	20.76	23.88

Table II. presents data typical of many experiments carried out with skins in the "normal" direction over 16 mm. tubes. They were washed for fifty minutes in the respective salt solutions and then immersed in 5 per cent. urea dissolved in the same salt solutions as used on the inside of the tube. Penetration continued four hours.

Table III. gives data obtained with the skins in the reverse relation, *i.e.*, "turned," over 16 mm. tubes. The skins were washed for one hour in the respective solutions before the experiment started and were then immersed in 5 per cent. urea dissolved in the same isotonic salt solution as was used inside the tube in each case. The penetration continued five hours.

Table II.

Date of experiment 11/3/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	13.32	13.68
10	90	11.88	16.62
15	85	14.04	18.90
25	75	12.60	16.08
50	50	11.16	15.24
100	0	12.66	11.82

Table III.

Date of experiment 11/11/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	5.88	14.04
10	90	1.44	5.28
15	85	1.26	6.48
25	75	3.90	9.18
50	50	4.56	7.44
100	0	6.72	4.44

Table IV. is another typical series with the skins "turned" over 16 mm. tubes. The skins were washed for one hour in the respective salt solutions and then immersed in 5 per cent. urea dissolved in water. Penetration continued for three hours and twenty minutes.

Table IV.

Date of experiment 12/12/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	6.72	8.28
10	90	9.84	9.18
15	85	7.68	10.62
25	75	3.66	7.08
50	50	4.26	7.44
100	0	2.04	5.76

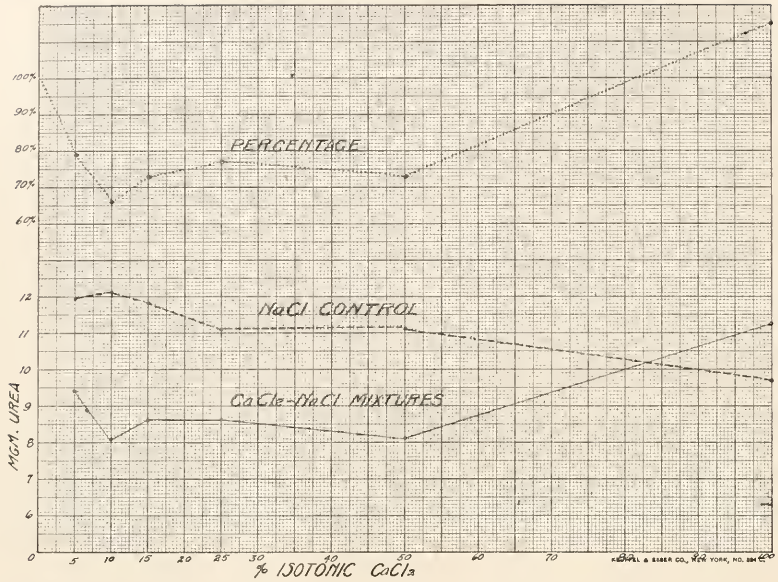

CHART I. The chart represents the composite results obtained in ten experiments.

The solid line represents the amount of urea in milligrams penetrating the skins in the $CaCl_2$-NaCl mixtures.

The broken line represents the amount of urea in milligrams penetrating the skins in the control experiments when NaCl solution alone was used.

The dotted line represents the percentage of permeability on the basis of the permeability with pure 0.7 per cent. NaCl solution alone, which is taken to be 100 per cent.

The minimum penetration of urea is obtained with very different percentages of $CaCl_2$ in the various series of experiments. This might be expected from the variability of the tissue to which reference has already been made. With a material like frog skin, composed of many layers of cells, there can be obtained only a rough approximation of the actual proportions of salts in contact with the majority of cells. When one is using skin comparatively permeable to the salts in question, and has washed it in the salt solution for some time before subjecting it to the penetration of urea, the proportions of salts in contact with the cells in the interior of the tissue approach more closely to those of the original solution than is the case at other times. With tissues relatively impermeable to the salts, it takes a higher concentration of $CaCl_2$ in the external solution to produce a concentration of about 10 per cent. in the region of the greater number of the cells. In support of this idea the figures presented show that sometimes the minimum amount of urea penetration was obtained with 25 per cent. or 50 per cent. $CaCl_2$, or even in one case in pure isotonic $CaCl_2$ solution, as in Table IV., quoted. When the urea was dissolved in the same mixture of isotonic salt solution as that used on the other side of the skin, the concentration of $CaCl_2$ within the inner strata of the skin was probably much more easily reached, and the most striking differences in the permeability to urea was shown with experiments thus carried out.

Summary and Conclusions.

Experiments were carried out on the effects of NaCl and $CaCl_2$ alone and in combination upon the permeability of frog skin to urea, with the following results.

Frog skins bathed in isotonic solutions containing varying proportions of $CaCl_2$ and NaCl showed less permeability to urea than did the controls of matched skins bathed in pure isotonic NaCl.

The minimum amount of penetration of urea, while varying much with different skins, was obtained on an average with about 10 per cent. of a 1.1 per cent. $CaCl_2$ solution plus 90 per cent. of a 0.7 per cent. NaCl solution.

In many cases, isotonic $CaCl_2$ solutions alone as compared with pure NaCl solutions increased the permeability to urea.

The writer is indebted to Dr. M. H. Jacobs for suggesting the study of urea with frog skin and for his continued interest and criticism.

This work was first undertaken at the University of Pennsylvania during the year of 1922–23, but could not be completed until facilities were obtainable again at the University of Texas during 1925–26. This accounts for the long break in the date of the experiments.

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THE PERMEABILITY OF FROG SKIN TO UREA.

II. The Effect of Dextrose and Sucrose.

M. L. BASKERVILL.[1]

Introduction.

Loeb (1) in a series of papers on the passage of potassium through the membranes of *Fundulus* eggs demonstrated that this substance would pass through in the presence of other electrolytes, but failed to do so with various non-electrolytes. The explanation that he offered and termed the "salt effect" could apply only to electrolytes.

Since the experiments of Loeb on potassium, there has been a good deal of other work comparing the permeability of tissues to electrolytes in the presence of other electrolytes and in the presence of non-electrolytes. Hoeber and Memmesheimer (2) showed that basic dyes enter red blood corpuscles if dissolved in salt solutions, but not if they are dissolved in sugar or glycocoll solution. Wertheimer (3) confirmed the need of electrolytes for the penetration of basic dyes through frog skin. Hiruma (4) reported that blood corpuscles take up less ammonium and alkaloid salts from a sugar solution than from a salt solution, while more salicylate or thiocyanate is taken up from the sugar solution. With frog muscle he found that basic dyes, alkaloid salts, salicylate, and thiocyanate have their penetration favored by sucrose as have also iodide and acid. The sugar checked only sodium hydroxide.

Embden and Adler (5) noted that if frog muscles were immersed in isotonic sugar solution, phosphoric acid would pass out, and the muscles would lose their contractility, although even after a stay of several hours in the sugar solution they would recover upon being again placed in Ringer's solution. Phosphoric acid was also given off in Ringer's solution if the muscles were fatigued or injured. The authors believed there was a direct connection between the permeability for phosphoric acid and a non-functional, *i.e.*, an injured, condition of the muscle.

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Among the papers describing the conditions for the permeability of non-electrolytes, probably no other is so free from the objection that the permeability reported might be due to injury as an article by Reid (6) in which he found that absorption of glucose from the intestine is favored by the presence of salts. Hiruma (4) in the paper referred to above reported that the permeability of blood corpuscles for urea is not decreased but probably increased by the presence of other non-electrolytes.

Wertheimer (3) claimed that if frog skin had its inner surface in contact with solutions of non-electrolytes more glucose would penetrate than was the case when Ringer's solution was employed in the same way. In an earlier paper (7) he had reported that sugar passed through the frog skin into Ringer's solution if the sugar was in contact with the inner surface of the skin and the Ringer's solution on the outer surface, while no sugar passed if the direction of the skin was reversed. The durations of the experiments were fifteen hours or more. Bauer (8) objected that Wertheimer secured more permeability to sugar under the conditions that are more injurious to the skin; that is, with sugar in contact with the inner surface of the skin. In his last paper Wertheimer (9) has again stated that if a non-electrolyte bathes the inner surface, frog skin is permeable to sugar; if an electrolyte bathes the inner surface, it is impermeable. This is just the condition that Bauer maintains is most injurious, so some evidence as to the state of the skins is needed.

Most of the writers mentioned confirm the influence of electrolytes in favoring the permeability of tissues to other electrolytes. The evidence in regard to their effect on the permeability of tissues to non-electrolytes is both more meagre and more conflicting. Since the permeability of frog skin to urea has been shown in the preceding paper (10) to be decidedly influenced by the kind of salts in contact with the tissues, the writer wished to see what effect the absence of electrolytes, or the presence of another non-electrolyte would have on the penetration of the non-electrolyte, urea.

The permeability of frog skin to urea as influenced by electrolytes and by non-electrolytes may readily be ascertained, since direct chemical determinations of the amount of the substance passing through the skin may be made. Urea has the further advantage of penetrating so readily that the tissues may maintain their vitality through the period of experimentation. Hence it seemed of interest to find how much urea will pass through frog skin when it is bathed in Ringer's solution and when it is bathed in sugar solutions.

In experiments carried out during the winter of 1922–23 much less urea penetrated into sucrose solutions than into Ringer's solution. As soon as facilities for the work could be secured again, the experiments were repeated and extended to dextrose. While the results quoted are in most cases the more recent ones, they do not differ in kind from those obtained several years previously.

Materials and Methods.

The procedure was similar to that described in the foregoing paper (10). Matched pairs of frog skin were put over 16 mm. tubes. Into one tube of each pair was measured a convenient amount, usually 2.5 cc. of the sugar solution; into the other, the same amount of Ringer's solution. Each tube was then placed with its other surface bathed by the urea solution. All solutions referred to were isotonic except the urea solution. which was five per cent. In preparation for the experiment, each skin was usually washed about an hour in the particular solution that was to be used on the inside of the tube. The exposure to urea was continued for varying times. Series of experiments were carried out with both dextrose and sucrose.

When the study of the permeability of frog skin to urea was begun, much time was spent in trying to discover if the orientation of the skin made any difference in its permeability to urea. Przylecki (11) has reported that urea penetrates frog skin equally well in both directions. Under the conditions employed in this paper it was found to penetrate readily in either direction, while no marked difference in the rate of penetration in the two directions was shown except in tubes where a solution used might be more injurious to one side of the skin than to the other. For example, if sugar solution was in contact with the inner surface of the skin, after five or six hours urea penetrated much more rapidly from the outer surface than was the case if the reverse relations held. For this reason, while results are reported below for both directions, it is considered that the more significant ones were obtained with skins that had their inner surfaces in contact with urea solution, which seems fairly harmless for the tissue, and the outer surface in contact with the sugar solution. The use of solutions abnormal for the skins is discussed in detail in the recent article by Bauer (8).

DATA.

Throughout the tables "normal" direction indicates that the inner surface of the skin was toward the tube and hence in contact with the sugar or Ringer's solution respectively, and the outer surface in contact with the urea; while "turned" indicates the reverse relation. The amount of urea penetrating was determined by the Van Slyke-Cullen method, and is expressed in milligrams in all tables. The figures for matched skins are given in parallel columns.

The data outlined in Table I. show the kind of results always obtained when pure sugar solution and Ringer's solution were compared with the skins turned in the two directions. The skins were washed forty minutes in Ringer's or dextrose solution, respectively. Penetration was allowed to continue for three hours and forty minutes.

TABLE I.

4/2/23.

	Isotonic Dextrose Solution.	Ringer's Solution.
	Skins "Normal" Direction.	
	Mg. Urea.	Mg. Urea.
	5.88	9.72
	4.50	9.00
	5.40	10.20
Average	5.26	9.84
	Skins "Turned."	
	2.16	14.46
	2.46	15.60
	2.40	17.52
Average	2.34	15.86

In Tables II. and III. are presented typical data showing the penetration of urea dissolved in varying concentrations of dextrose. The Ringer's and dextrose solutions were in contact with the inner surfaces. The percentage of isotonic sugar solution is indicated in the first column; it is made up to 100 cc. with Ringer's solution in each case. The skins were washed for one hour in the respective solutions. Penetration was allowed to continue for three hours. The skins were "turned."

TABLE II.

11/30/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	7.32	7.86
75	5.16	7.44
85	5.22	8.34
90	7.50	7.86
95	1.68	9.48
100	0.96	8.04

TABLE III.

12/1/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	3.72	5.88
75	2.76	7.74
85	4.08	6.66
90	4.80	7.02
95	2.64	8.70
100	1.20	4.74

Results with the skins in the reverse relation are given in Tables IV. and V. The skins had the inner surface in contact with the Ringer's or sugar solutions, and the outer side in contact with five per cent. urea. The skins were washed for one hour in the respective solutions. Penetration continued for three hours, and the skins were in the "normal" direction.

The results with sucrose differed no more from those obtained with dextrose than did results with either sugar alone when different skins were employed, as is indicated by the data recorded in Tables VI. and VII. The skins were "turned" and washed for one hour in the respective solutions. The time of penetration was three hours in all cases.

Table IV.

12/3/25.

	Isotonic Dextrose + Ringer's Solution.	Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	5.10	5.28
75	4.32	7.80
85	2.76	4.86
90	2.52	3.96
95	4.20	5.64
100	2.34	5.76

Table V.

12/4/25.

	Isotonic Dextrose + Ringer's Solution.	Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	7.08	6.12
75	4.50	8.10
85	6.12	6.48
90	6.88	6.90
95	5.82	7.80
100	4.44	7.74

Table VI.

3/2/26.

	Isotonic Sucrose + Ringer's Solution.	Ringer's Solution.
Per Cent. Sucrose.	Mg. Urea.	Mg. Urea.
50	7.26	9.72
75	8.46	9.06
85	6.00	8.58
90	2.34	9.06
95	3.96	8.34
100	0.30	8.22

Table VII.

3/1/26.

	Isotonic Sucrose + Ringer's Solution.	Ringer's Solution.
Per Cent. Sucrose.	Mg. Urea.	Mg. Urea.
50	6.24	6.84
75	9.12	10.50
85	7.02	8.58
90	5.70	9.18
95	4.98	10.68
100	0.54	8.76

Reference has been made to the greater permeability to urea in sugar solutions if the exposure is prolonged beyond three or four hours. Table VIII. contains typical results showing more penetration of urea in the solution of pure dextrose than in the

TABLE VIII.

2/27/25.

Isotonic Dextrose + Ringer's Solution. Per Cent. Dextrose.	Isotonic Dextrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	4.14	6.60
75	3.30	9.72
85	4.80	7.98
90	3.18	3.18
95	4.20	4.98
100	10.32	6.66

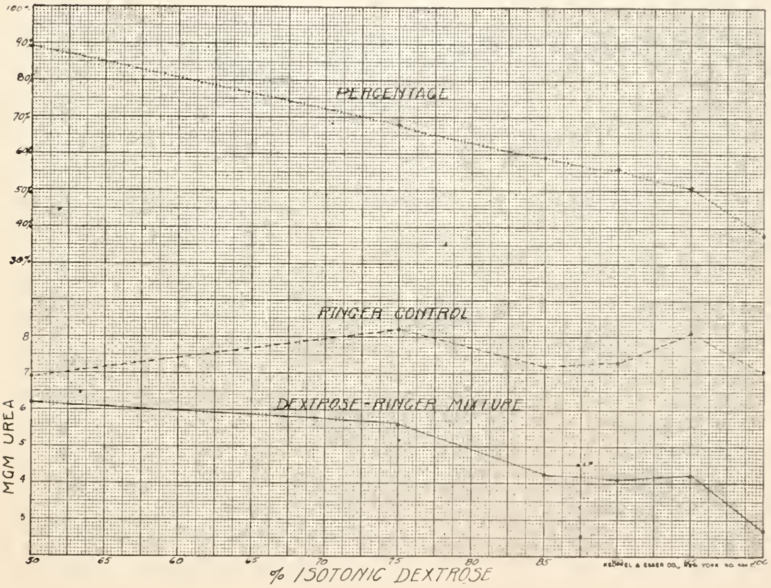

CHART I. The chart represents the composite results obtained in ten experiments.

The solid line represents the amount of urea in milligrams penetrating the skins in the Dextrose-Ringer's mixtures.

The broken line represents the amount of urea in milligrams penetrating the skins in the control experiments when Ringer's solution alone was used.

The dotted line represents the percentage of permeability on the basis of the permeability with Ringer's solution alone, which is taken to be 100 per cent.

control of Ringer's solution; and less penetration than into the controls in solutions containing dextrose with sufficient amounts of Ringer's to protect the skin against too rapid death. The skins were in the "normal" direction; washed for one hour and forty-five minutes; penetration five hours.

In order to compare the penetration of urea through dead frog skin with that through living skin, the skins were killed in several ways: by exposing to 60 per cent. ethyl alcohol; to 5 per cent. formalin; or to 0.5 per cent. sodium fluoride, from forty-five minutes to one hour. Often skin that had been killed in alcohol or sodium fluoride showed the passage of more urea into the pure sugar solution than into Ringer's solution. The reason for this behavior is not known, unless it is that the constituents of the skin are not "fixed" so thoroughly by these chemicals as by formalin, and therefore the subsequent treatment with sugar causes more disorganization in the skin and more penetration than could occur in the latter case. This point is of chief interest in showing that the more viscous sugar solutions do not slow the rate of diffusion of urea more than does the Ringer's solution.

In Tables IX. to XII. are presented data typical of results obtained in a large number of experiments with dead skins. The skins were washed in the sugar or Ringer's solution respectively before and again after being killed in the solutions indicated. Penetration of the urea continued from three to three and a half hours.

Table IX.

3/19/26.

	Isotonic Sucrose + Ringer's Solution.		Ringer's Solution. Mg. Urea.
	Per Cent. Sucrose.	Mg. Urea.	
Killed in alcohol.			
"Normal" direction	50	15.66	10.68
	75	18.90	10.89
	100	21.12	10.50
"Turned"	50	15.99	9.51
	75	19.17	10.59
	100	19.71	11.16

TABLE X.

4/27/26.

	Isotonic Sucrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	1.74	2.07
	1.11	1.98
Killed in alcohol:		
"Normal" direction	19.83	12.63
"Turned"	17.49	11.58
Killed in NaF:		
"Normal" direction	5.82	5.04
"Turned"	6.45	4.95

TABLE XI.

5/12/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Turned"	4.56	7.32
Killed in NaF:		
"Normal" direction	4.32	1.98
"Turned"	3.84	3.78
Killed in formalin:		
"Normal" direction	7.56	7.74
"Turned"	8.16	6.84
Killed in alcohol:		
"Normal" direction	24.48	18.48
"Turned"	26.82	21.78

TABLE XII.

5/18/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	3.54	5.16
Killed in NaF:		
"Normal" direction	12.74	10.08
"Turned"	5.58	3.84
Killed in formalin:		
"Normal" direction	3.48	3.00
"Turned"	3.90	5.46
Killed in alcohol:		
"Normal" direction	14.76	14.76
"Turned"	18.72	15.30

Discussion.

The experiments show that in the case of the shorter exposures the passage of urea through frog skin was checked by the presence of sugar. After about six hours, the time differing with different skins, the skins in a pure sugar solution were greatly injured, especially if the sugar was in contact with their inner surfaces. As a rule, skins in mixtures of sugar and salt solutions survived in proportion to the amount of salt present. In contrast to these conditions, skins bathed on the inner surface by Ringer's solution survived 24 hours and upward. Accordingly, when skins were exposed to the solutions for six hours or more, the skins in contact with the solutions containing the larger proportions of sugar were the more injured and permitted more urea to pass. Skins that had been killed in various ways showed more nearly the same degree of permeability in Ringer's and in sugar solutions.

As in all work on permeability of cells and tissues, it is necessary to distinguish carefully between the permeability caused by injury and that normal to tissues. It is not claimed that in these experiments the skins remained entirely uninjured during the time—three or four hours—that constituted the minimum exposure to the solutions used, which are quite abnormal for frog skin; but it is believed that the skins remained in a sufficiently healthy condition for this length of time to exercise a considerable degree of the selective permeability that is characteristic of living tissues. In spite of the fact that skins exposed to the sugar solutions even for the shorter periods would be expected to be proportionately more injured than those exposed to Ringer's solutions, more permeability to urea was shown in the latter condition.

Because of these results on urea it may be concluded, therefore, that the favorable effects of salts on the penetration of dissolved substances is not limited to the passage of other electrolytes as some authors have assumed, but applies to certain non-electrolytes as well. This is not surprising considering the universal presence and the known physiological importance of the electrolytes in living cells and tissues.

SUMMARY.

1. Isotonic dextrose or sucrose solutions, as compared with Ringer's solution, check the passage of urea through frog skins if the exposure to the solutions is not so prolonged that the skins are injured.

2. When the skins are kept in contact with the solutions for such a length of time that those in the sugar solutions are injured or killed and those in Ringer's solution are still in a healthy state, then more urea may penetrate in the presence of the sugar solution.

3. Mixtures of isotonic sugar solution and Ringer's solution check the penetration of urea in proportion to the amount of sugar present when that amount reaches a concentration of 50 per cent. or more.

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THE CLOACA AND SPERMATHECA OF *GYRINOPHILUS PORPHYRITICUS*.[1]

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Introductory.

The first observations on the cloaca and spermatheca in American urodeles were made by Kingsbury in 1895. He described seven forms. Apparently the development of the spermatheca did not receive attention until recently, when Koehring ('25) described it in *Eurycea bislineata*. In the present study a description of the form and development of the cloaca and spermatheca in *Gyrinophilus porphyriticus* has been attempted, and certain observations have been made which throw some light on the question of time of mating and ovulation in this form.

The material consists of fourteen fully developed adults, six animals which had undergone metamorphosis but were not fully developed, and six larvæ. After fixation in Bouin's fluid, formalin or alcohol, the cloacæ of all the animals except one adult were imbedded in paraffin and sectioned serially, usually at 12 μ. The remaining adult was studied under the binocular dissecting microscope. The ovaries of eleven animals were sectioned to determine size of ova and deposition of yolk at different seasons. A single specimen each of *Eurycea*, *Plethodon glutinosus*, *Plethodon cinerius*, and *Diemyctylus* were sectioned for comparative study.

Observations.

The Cloaca in Adults.—The mid-sagittal section (Fig. 11) shows the main features of the cloaca, namely, a well marked diverticulum located in the dorsal wall above the vent, ahead of this a narrowed portion, which, as it is followed in a cephalic

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[1] I desire to express my gratitude for advice and guidance, as well as for material, to Dr. R. R. Humphrey, who suggested this study.

direction, expands both dorsally and ventrally. In the dorsal recess thus formed, the uro-genital papillæ are located; at the same level the cloaca communicates ventrally with the bladder.

The cloaca is a slit-like cavity, and is thrown into well-marked folds, of which, however, only those on the dorsal wall are significant. In the middle third of the cloaca there are two folds in this location, with a median dorsal slit between them (Figs. 4 and 5). The slit extends as far forward as the opening of the spermatheca (Fig. 6), cephalic to which the two folds are fused into one (Fig. 7). The folds, including both their distinct and fused portions, correspond to what Kingsbury (1895) has called in other species, the dorsal elevation. The extent of the elevation is indicated on the sagittal section (Fig. 11). The shelf-like area seen below the diverticulum in the same section corresponds to the freely projecting papilla described by Kingsbury in *Plethodon;* in *Gyrinophilus*, however, it is not free like a tongue, but is merely the dorsal wall of the cloaca below the diverticulum, and is continuous across from one lateral wall to the other. Since the mid-sagittal section passes between the two limbs which compose the caudal two thirds of the dorsal elevation, these limbs or folds do not appear in this section.

Cephalic to the fused portion of the dorsal elevation, this structure is seen to break up into three folds (Fig. 8), the lateral of which merge into the lateral cloacal walls, while the median, a slender fold, after continuing forward a short distance (Fig. 9), disappears abruptly as the cloaca expands dorso-ventrally. The entire elevation is pigmented, especially in the region of its two limbs caudal to the opening of the spermatheca.

According to Kingsbury, both dorsal and ventral glands may be present in the cloaca of the urodele female; but they are quite variable, for, of seven forms described by him, *Diemyctylus* has ventral glands only, *Necturus* and *Amblystoma* have both well developed, *Eurycea* has ventral well developed and dorsal rudimentary, and the *Plethodons* and *Desmognathus* have neither. In *Gyrinophilus* females the ventral glands alone are well developed. They lie, one group on each side of the mid-line, in the ventral and lateral walls of the cloaca (Fig. 4). The area occupied by glands extends from the cephalic end of the dorsal diverticulum caudally, to the opening of the spermathecal tube cephalicly, or approximately between the levels of Figs. 4 and 5 indicated on the sagittal section (Fig. 11). The glands are tubular in form and somewhat tortuous. Their epithelium is of the mucous-secreting type. Their narrow ducts open into the ventral and lateral walls of the cloaca, both on the summits of and between folds.

In addition to these functional glands, a group of rudimentary glands is found, opening into the mid-dorsal slit, caudal to the spermatheca. These are inconstant and variable in number. They are sometimes absent; sometimes only a single tubule is found on one side, or there may be three or four pairs.

The epithelium of the cloaca is a stratified squamous just inside of the vent, throughout the caudal portion of the cloaca, in the dorsal diverticulum, and on the elevation as far forward as the opening of the spermatheca. This gives way to a stratified cuboidal epithelium, which is two layers deep on the ventral wall of the cloaca opposite the spermathecal tube, and three or four layers on the elevation, in the median slit, and in the cephalic expansion of the cloaca. This stratified epithelium, in turn, gives way in the gut to a tall columnar variety with goblet cells, and in the bladder to a simple cuboidal.

A comparison of the cloaca of *Gyrinophilus* with that of other forms, relates it most closely to *Eurycea* and the Plethodons. *Eurycea*, though lacking the diverticulum, has the dorsal elevation divided into two limbs with the median dorsal slit between them; and the Plethodons, in which species the elevation has no dorsal slit but is single throughout, have the dorsal diverticulum. In *Plethodon glutinosus*, this diverticulum extends forward above (dorsal to) the spermathecal region in my specimen. Kingsbury describes it as of considerable extent.

The Spermatheca in Adults.—The spermatheca, or sperm-storing organ, definitely associated in urodeles with internal fertilization, is, in *Gyrinophilus*, a complex structure, located dorsal to the cloaca, and consisting of a branched common tube and numerous smaller tubules. The common tube, constricted slightly near its mouth, and pointing almost directly dorsad from its opening between the limbs of the dorsal elevation,

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expands into two short but thick lateral branches. These branches curve both latero-ventrad and caudad (Figs. 5 and 12). Into them and into the distal end of the common tube open small flask-shaped tubules, with long slender necks and bulbous, blind ends; these are bilaterally arranged in two groups, varying from fifteen to twenty-five in each group in different animals. Rarely a tubule is double: *i.e.*, one slender neck terminates in two bulbs. The tubules point caudad, dorso-caudad, laterad and ventrad, but not cephalad and not directly dorsad. Spermatozoa, when abundant, are found in the common tube, its branches, and in both neck and bulb of the tubules (Fig. 12). In the bulbs they are arranged in a whorl (Fig. 13), lying side by side, heads all pointing in the same direction. A similar cluster is sometimes found streaming into the neck of a tubule, but in other necks and in the common tube, there are only a few stray sperms. When sperms are scanty, they are tangled and disordered, as though they had been brushed off when the larger clump was expelled by muscular contraction, and are found in the lateral branches of the common tube, and in some tubules, but are absent from others.

Surrounding the organ as a whole, as well as each tubule and the common tube, are numerous plain muscle fibers. Pigment cells, containing coarse black or brown granules, are found to be most abundant around the expanded end of each tubule, moderately abundant in the dorsal elevation, and scanty beneath the epithelium of the rest of the cloaca, among the muscle fibers of its wall, and about the ureters.

The epithelium of the spermatheca is stratified in the common tube and its branches, the surface layer of columnar cells becoming progressively taller toward the upper end of the tube. It becomes a simple, low columnar in the necks of the tubules, and a simple, tall columnar in their expanded ends. In this location the cytoplasm takes a deeper eosin stain than in any other part of the organ, and is finely granular.

The size of the spermatheca in animals of 16.5 cm. length is approximately 1.05 to 1.20 mm. in each of its dimensions (transverse, cephalo-caudal and dorso-ventral, including the common tube). If larger, it is the transverse diameter which is most likely to be increased. In the smallest adult in the series, measuring 12.0 cm., the dimensions were 1.0 mm. each. The above measurements were made from serial sections of the organ.

Discussion.

An examination of the spermatheca in different species suggests that the complex forms found in *Eurycea*, *Gyrinophilus*, the Plethodons and *Desmognathus* are developed from the simpler forms (Kingsbury). In *Diemyctylus* and *Necturus* each tubule opens separately into, or along the margin of a dorsal depression or slit in the cloaca; this slit in *Diemyctylus* is partially divided into two by a low ridge. The tubules are numerous (25–40), and flask-shaped. In *Amblystoma* the tubules open, not into a dorsal slit, but into a depression in the dorsal wall, and instead of being scattered, are aggregated and surrounded by plain muscle. *Gyrinophilus*, retaining the numerous tubules, presents, instead of a depression, a tube, constricted at its neck. Its branching reminds one of the definitely bilateral character of the organ in *Diemyctylus*. *Eurycea*, with its occasionally branched [1] common tube, resembles *Gyrinophilus*, but has fewer tubules (12–14). The Plethodons and *Desmognathus* present a further reduction in number of tubules (4–6), and a loss of the characteristic flask shape.

If these forms be arranged in a phylogenetic series on the basis of this one feature, the form of the spermatheca, the tendency in the ascending scale seems to be, first, the aggregation of the tubules from a scattered position along a dorsal slit, to a group around a common tube, the expanded end of which may or may not be branched; and second, the reduction of the number of tubules and loss of their flask shape. Correlated with these tendencies in the spermatheca, the importance of the glands is diminished. If we except *Diemyctylus*, the lower members (*Necturus* and *Amblystoma*) have both dorsal and ventral glands, though Kingsbury states that the epithelium of the dorsal glands of *Necturus* resembles that of the cloaca, rather than that of other cloacal glands. *Eurycea* and *Gyrinophilus*, which stand mid-way in the series, have functional ventral glands, and rudimentary dorsal glands. The Plethodons and *Desmognathus*, at the top of the series, have neither dorsal nor ventral glands. Kingsbury suggests that spermathecal tubules are modified dorsal glands. Apparently, as the spermatheca departs farther from the simple tubules from which it was derived, the gland tubules are suppressed; the dorsal group first, and later the ventral.

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[1] Kingsbury does not describe the branching of the common tube in *Eurycea*, but I find it in my specimen, and Koehring, who finds it only in her largest animal (90 mm.), regards it as a late development. Possibly the occasional occurrence of branching in this form represents a transition between the branched common tube of *Gyrinophilus* and the unbranched of the Plethodons.

The possible significance of pigment cells as attracting or being attracted by spermatazoa is discussed by Kingsbury. There appears to be some correlation between the pigmentation of the spermatheca in the female and the testes and ducts in the male,[1] for *Eurycea, Gyrinophilus*, the Plethodons and *Desmognathus*, all show abundant pigmentation in both sexes; *Amblystoma* none in either sex; *Diemyctylus* moderate in the female, and little in the male. In *Necturus* the spermatheca is free from pigment, and testes and ducts only very slightly pigmented.

Pigment is absent from the cloaca of *Gyrinophilus* in the earliest stages of development of the spermatheca, but appears in increasing amount with advancing development. In this series it is found in fair abundance in a small female with well developed spermatheca. The size of this animal (12 cm.), the absence of debris in the organ, and the minuteness of the ova speak against the animal ever having mated. We may conclude, therefore, that the deposition of pigment is not in response to the presence of sperms, but is a normal developmental phenomenon, and may serve to attract sperms, as suggested by Kingsbury.

Three questions naturally suggest themselves in a study of the spermatheca: first, the probable time of mating, second, the time of ovulation, and third, the length of time sperms may remain in the organ. The following generalizations, drawn from facts tabulated in Table I., have some bearing on these problems in *Gyrinophilus:*

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[1] Information regarding pigmentation in the males of these species is supplied by Dr. R. R. Humphrey.

TABLE I.*

SHOWING PRESENCE OF SPERMS AND SIZE OF OVA IN *Gyrinophilus* FEMALES AT VARIOUS MONTHS.

(The males have no sperms in the vasa deferentia from approximately June 1 to September 15.)

In the following table (1) signifies that the cloaca, or ovary, was not available for examination; (2) that, although the ovary was not available, the ova were recorded as "minute" at the time of capture of the animal; (3) that two animals of the same size were captured on the same date, and gave identical findings; + that the tail of the animal was broken, and the actual length was greater than that given.

Date.	Presence of Sperms in Spermatheca.	Size of Largest Ova in Mm., and Deposition of Yolk.	Length of Animal in Cm.
Mar......	(1)	0.41; yolk absent	16.7
Apr. 6..	Absent (spring captivity)	2.50; " abundant	16.5
June 19..	Absent	0.68; " scanty	14.3 +
June 26..	Abundant	2.75; " abundant	17.0 +
July 1..	Moderate	0.97; " moderate	16.5(3)
July 1..	Scanty	(1)	15.5
July 24..	(1)	0.32; yolk absent	(1)
July 25..	Absent	(1)	14.5
Aug. 15..	Abundant	2.70; yolk abundant	13.5
Aug. 15..	Absent	1.00; " scanty	13.3
Sept. 25..	Scanty	1.7 ; " abundant	16.5
Sept. 26..	Absent	(2)	12.0
Nov. 19..	Abundant	2.25; yolk abundant	17.5
Nov. 19..	Absent	(2)	15.5

1. The smallest animal in which sperms or large ova were found, measured 13.5 cm.; therefore, only animals of this size and larger were considered in studying the question of mating and ovulation. In smaller animals (12.0 and 13.3 cm.), the spermatheca, though well developed, was found perfectly clean and free from debris.

2. The presence of sperms in the spermatheca does not depend upon season, for they were abundant in June, August and November specimens (one in each month), moderate or scanty in July, scanty or absent in September, and absent in some animals in every month in which any specimens were captured.

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*Two additional animals have been examined, both killed in May. The first, measuring 15.3 cm., was captured and killed May 2d. Ova measured 1.0 mm., yolk was scanty, sperms were absent. The second was longer than 15.0 cm., and was killed May 3rd after two weeks of captivity with a male. Ova were minute, yolk absent, and sperms absent.

3. Ova, presumably large enough for ovulation (2.25–2.75 mm.), appeared in April, June, August and November. Since the August animal was small and possibly ovulating for the first time, the other three might point to spring and fall ovulations in this species; but there is no sequence in size of ova leading up to April, June, or November. Possibly each animal ovulates but once a year, and food, temperature and individual habit may determine the time.

4. Of the four animals showing large ova, sperms were abundant in the three taken in June, August and November. The fourth animal, killed in April, had been kept in captivity for several weeks, and would thus have been prevented from mating during that period.

5. Sperms were not found in abundance except in animals nearly ready to ovulate. Whether or not normal sperms might be retained long enough to fertilize ova at the following ovulation, would be impossible to say from this series. An animal taken in September with ova two thirds of the maximum size (ready to ovulate in October or November?) had a few sperms remaining.

6. Mature sperms are absent from the vasa deferentia of the male from approximately June 1 to September 15.[1] Males are, therefore, incapable of functional mating during this period. The sperms found in females between June and September must have been acquired before June—probably in early spring or the preceding autumn. Of especial interest is the abundance of apparently normal sperms in the female taken in August. We may conclude that sperms can be stored in the spermatheca for at least two and a half months, and most probably for a far longer period.

The interesting questions of mating and ovulation cannot be answered without further study; we may conclude that both phenomena are probably quite variable as to time, that sperms are stored in the spermatheca from mating until the succeeding ovulation, and that apparently normal sperms are found for a considerable time after ovulation.

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[1] This information is supplied by Dr. R. R. Humphrey. He has examined males killed in every month except April.

Development of the Spermatheca and Cloaca.

The cloaca in larvæ ranging in size from 7.7 to 10.0 cm. is very simple; there are few folds in its lateral wall and these disappear on distention of the cloaca. One, however, in the dorsal wall, persists in spite of distention. It is located just caudal to the dorso-ventral expansion into which open ureters, oviducts, gut and bladder. Because of its location, its slenderness, and its delicate epithelium, this fold is interpreted as the medial of the three folds into which the dorsal elevation divides in the adult (Figs. 14, 8, and 9). Caudal to this in the roof of the cloaca, the epithelium grows very much thicker and has a well-defined row of basal cells and basement membrane (Fig. 15).

In two larvæ of 10.3 and 10.5 cm. respectively, a noteworthy change is observable. Over a short area caudal to the slender fold described above, and dorsal to the vent, the basal row of nuclei is not distinct, and the nuclei of the epithelium are small and numerous. The overlying tissue is likewise richly cellular, and is mesenchymal in character. Upon superficial examination the nuclei of epithelium and overlying tissue are indistinguishable and seem to intermingle. Even upon closer examination they are quite similar, but a basement membrane, though very delicate, is apparent. The epithelium of this area, which stands in clear contrast to that of the rest of the cloaca, has been termed for convenience, the "cellular area," and is regarded as the anlage of the spermatheca (Fig. 16). The epithelial cells of the area show a distinct tendency to arrange themselves in rows perpendicular to the surface. Spaces often appear between the rows, but might be interpreted as artefacts. In one place a small bulb of cells has formed within the epithelium (Fig. 16*a*). Fig. 17*a* shows an early stage in the development of dorsal glands from the same animal. It will be seen that the gland tubule is in a slightly more advanced stage than the tiny bulb of cells in the cellular area. Both gland and spermathecal tubules are epithelial outgrowths.

One would probably not be justified in interpreting the picture seen in the cellular area, if taken alone, as an invasion of epithelial cells into overlying tissue were it not for the unequivocal evidence of such invasion discernible in the next stage. (The term "invasion" is used in the embryologic sense, as a downgrowth of a bud or cord of cells of epithelial origin, not in the pathologic sense implying unrestrained growth with loss of basement membrane.)

Glands in the larva of 10.5 cm. have been referred to above. There are two groups of these; the tubules, as shown (Fig. 17 and 17*a*) are very tiny and most of them are still solid cords. The dorsal group is slightly better developed than the ventral. The former, located caudal to the cellular area, is bilateral; its tubules, numbering four to six on each side, are outgrowths from the epithelium of the roof of the cloaca, and point dorsolaterad. A ventral group of about the same number, is located cephalad to the level of the cellular area; these are outgrowths from the epithelium of the extreme ventral portion of the cloaca.

Next in the series of stages is the smallest unmistakable spermatheca, in an animal measuring 10.8 cm., captured in May; a small stub of gill left on one side gives evidence of recent transformation. It should be noted that this animal is further developed than the two previously described, although the latter were captured later in the summer (September and August respectively). This young spermatheca consists of a larger cellular area than that just described. The cells are arranged in definite rows or cords perpendicular to the surface, and most of the cords extending beyond the cellular area itself, terminate in an expanded bulb with a minute lumen. There are ten or twelve bulbs on each side, and they curve like plumes, dorsolaterad, laterad and cephalad. The less well developed tubules without definite bulbous expansions, are the most caudal of the group. The whole organ occupies less than 0.4 mm. Of especial interest is the lumen of the common tube. In its caudal portion it is pyramidal in cross-section, with the base of the pyramid dorsad (Fig. 18), and in its cephalic portion, rounded but irregular (Fig. 19). Caudally it merges into the dorsal slit; cephalicly it is replaced by the median fold which was observed in earlier stages. Both groups of glands noted previously are present. Though the ventral are but little more advanced in development, the dorsal are now small tubules with bulbous ends, similar in form to, but smaller than the spermathecal tubules. Their subsequent fate will be discussed.

It should be noted that the cellular area of the 10.3 and 10.5 cm. animals lies in the same relation to the two groups of glands and to the median dorsal fold, as does the spermatheca in the 10.8 cm. animal—namely, the ventral glands and the dorsal fold are more cephalad, and the dorsal glands are more caudad. This gives additional ground for interpreting the cellular area as the anlage of the spermatheca.

In the next stage (10.7 cm.) the whole organ has enlarged; the tubules which still spray both cephalad and laterad, are longer and more isolated from each other, and have definite lumina. Again the common tube is of interest; in its caudal portion its lumen is shaped like a short-stemmed Y, and has an epithelium three cells deep (Fig. 20); cephalad, however, the common tube is not branched, and several irregular lumina appear in the midst of the mass of cells from which the tubules spring, as though the common tube were enlarging by degeneration or pulling apart of epithelial cells as the tubules acquire lumina. Ventral glands are more numerous and open well up on the lateral walls of the cloaca. No dorsal glands are present in this animal.

A further development in an animal of approximately 11.0 cm. (tail partly broken off), is seen in the elongation and change of direction of the tubules; most of them stream conspicuously in a caudal direction and then curve ventrad (Fig. 22); others spray cephalo-laterad; while those most cephalic in position, point almost directly dorsad. In the adult there are no tubules pointing cephalo-laterad or dorsad; these have all been turned more caudad. In the 11.0 cm. animal the limbs of the Y-shaped portion of the common tube seem to be increasing in height by degeneration of epithelial cells where tubules spray out from the limbs of the Y (Fig. 23).

In the three animals just described (10.8, 10.7, and 11.0 cm.) the cloaca showed progressive increase in number and size of its folds, especially in the height and breadth of the pair lying one on each side of the median dorsal slit. These were first indicated by a slight notching in the lateral walls of the cloaca at the "cellular area" stage (Fig. 16), but developed rapidly (Figs. 18 and 20). This pair will subsequently become the caudal two thirds of the dorsal elevation in the adult. Moreover, the median dorsal slit into which the common tube opens has deepened slightly by the development of the folds which form the elevation. As a result of this, as well as because of the further invasion of the tubules and elongation of the common tube, the organ lies farther from the dorsal wall of the cloaca in the third specimen than in the first.

Ventral glands are not altered in the 11.0 cm. animal. Of the dorsal group, three are found on each side in their old position caudal to the spermatheca. The latter are rudimentary; the height of their development was apparently reached when the spermatheca first appeared. They are exceedingly variable in all adults as was noted previously. When present, they are always located a short distance caudal to the spermatheca. The ventral group, appearing at the same time, subsequently become long, tortuous tubules with epithelium of the mucous secreting type, and are the functional glands of the adult.

The final changes in the spermatheca consist of its further removal from the cloaca, the further bending of its tubules in a caudal direction, and a change in form of the common tube. By overgrowth of the dorsal wall caudal to the spermatheca, the caudal or Y-shaped portion of the common tube is lifted farther from the cloaca, loses its direct connection with the cloaca, and communicates with it only by means of the cephalic, unbranched portion of the common tube. Meanwhile, the branches of the common tube are drawn ventrally, so that the lumen of this portion of the tube, now most distal from the opening, comes to resemble an H (Fig. 25). They are also bent caudally, hence in their most distal (caudal) part, may be seen isolated from each other (Fig. 24). The unbranched portion of the common tube (formerly its cephalic portion), now considerably enlarged, leads from the cloacal wall to the cross-bar of the H, and to the two branches. This stage (Figs. 24, 25, and 26) is an intermediate one in development, and appears at 11.2 cm., but was also seen in one full sized adult of 14.5 cm. By further expansion of the cross-bar of the H, the expanded end of the adult common tube is formed; from this large chamber its two branches extend caudally and somewhat ventrally.

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The next striking changes in the cloaca are the final modification of the dorsal elevation to its adult form (cephalic portion fused, caudal portion divided), and the development of the diverticulum. The dorsal elevation, when last considered (11.0 cm.) consisted of two fairly well developed folds with the median dorsal slit between them. At the cephalic end of the slit, cephalic to the spermathecal opening, the slit was replaced by the slender median dorsal fold which has been a feature of the cloaca since early larval stages. In the 11.2 cm. animal the cellular condition of this slender fold, and its increase in breadth, seem to indicate hypertrophy. Gradually its caudal portion fuses with the two folds caudal to it; its cephalic portion remains slender, however. This fusion has been nearly completed at 13.3 cm., and fully at 13.5 cm. The hypertrophy is part of a general gradual overgrowth of the dorsal wall, which will be discussed further. By the process just described, the dorsal elevation of the adult is formed. It will be recalled that in the adult the elevation is divided in its caudal portion, but cephalic to the spermathecal opening, is fused; while still more cephalic the fused portion gives way to three folds, the median slender one, flanked by two smaller lateral ones.

The diverticulum is absent in all animals smaller than 11.2 cm.; it, likewise, is formed by the overgrowth of the dorsal wall previously mentioned. The diverticulum continues to increase in size as long as the animal grows, for in the largest animals its two prongs reach forward and partially flank the spermatheca.

Discussion.

The most interesting phenomenon in the cloaca of young *Gyrinophilus* females is the development of the spermatheca. The cellular area lying between the single mid-dorsal fold cephalicly and the dorsal glands caudally, has been interpreted in this study as its anlage. Even at this stage the mode of formation of its tubules is indicated by the arrangement of its cells perpendicular to the surface, and by a small bulb-like mass of cells apparently about to grow out into the overlying mesenchymal tissue. This invasion is very clearly shown in the next stage. Comparison of these spermathecal tubules with gland tubules shows that both originate in the same manner.

As the tubules grow into overlying tissue, the whole cellular mass likewise sinks somewhat, and a portion of the cloacal lumen is drawn into the cellular mass to form the common tube of the spermatheca. At this stage it is not yet a tube, but rather a shallow depression, pyramidal in cross-section in its caudal part, and communicates widely with the cloaca. In this way the phylogenetic history of the common tube is repeated. One is reminded that in *Amblystoma*, the first species in the phylogenetic series to have its tubules aggregated, the common chamber is a depression in the dorsal wall, and apparently merely a modified portion of the cloaca.

Even at this early stage, however, the common tube gives evidence of enlarging by degeneration of epithelial cells, a feature which is more marked in the following stages. From the first, the caudal part of the common tube indicates its subsequent branching. The caudal portion is next lifted away from the cloaca in a dorsad direction, and becomes the distal, branched portion of the common tube, communicating with the cloaca by means of the old cephalic portion, now drawn out into a long tube.

Koehring ('25) in describing the development of the spermatheca in *Eurycea*, says: "In a 58 mm. animal . . . there are four distinct tubules. . . . These are made up of a very small group of cells closely grouped and darkly stained with barely perceptible lumina and short ducts. The ducts very nearly reach the thick walls of an invagination of the cloacal wall which is the anlage of the central tubule." She does not specifically state in her paper that she regards the spermathecal tubules as epithelial downgrowths, derived from the cloacal epithelium lining the invagination or "central tubule" (common tube); and, since she says that the ducts "very nearly reach" the walls of the invagination, one might surmise that the tubules are formed in the overlying tissue and secondarily unite with the epithelium lining the invagination. If, however, it is the lumen of the duct which very nearly reaches the lumen of the invagination, this would coincide with the findings in *Gyrino-*

philus; in this form the tubules are at first solid outgrowths, the lumen developing first in the expanded distal portion, and later in its slender neck.

The common tube of the spermatheca in *Eurycea* is regarded by Koehring (see above) as a portion of the cloacal wall. This coincides with the findings in *Gyrinophilus.* It is possible that the "thick walls of the invagination" (Koehring) correspond to the "cellular area" described in the "first unmistakable spermatheca" in this study of *Gyrinophilus.* In this specimen the mass of epithelial cells from which the tubules spring, and into which a portion of the cloacal lumen has been drawn, is very thick. It is also difficult to distinguish the basement membrane which sets off the highly cellular tissue outside from the equally highly nucleated epithelium. Together they might be said to constitute a "thick wall" for the cloacal depression or common tube.

There are several features in the development of the cloaca and spermatheca which must be accounted for. I shall consider first the change in direction of the tubules to one which is chiefly caudad. Coincident with this change, the entire spermatheca shifts from a position dorsal to the vent, to one ahead of the cephalic end of the vent, while ventral glands come to lie caudal to the level of the spermatheca. Moreover, the cloaca is relatively and actually taller in the animal of 8.8 cm. (Figs. 14 and 15) than at 10.5 cm. (Figs. 16 and 17). All these facts point toward an elongation and flattening of the cloaca, coincident with increase in length of the whole animal. In Fig. 27 the outline of an adult cloaca, relatively reduced in size, has been superimposed upon the outline of a young cloaca. The long arrows, *a* and *b*, indicate the shifting of parts which would flatten the cloaca, bring ventral glands caudal to the level of the spermatheca, and bring the spermatheca cephalic to the vent. During the change of position of the spermatheca, its tubules are turned caudad.

Elongation of the cloaca, however, does not explain the development of the dorsal elevation, the fusion of its two limbs cephalic to the spermatheca, nor the development of the diverticulum. These are interpreted as being due to an overgrowth of the whole dorsal wall of the cloaca, as indicated in Fig. 27 by the small arrow ahead of the spermatheca, and the curved arrow behind it. This overgrowth would (1) elongate the common tube and lift its caudal portion farther from the cloaca; (2) form the dorsal elevation and cause the fusion of its two limbs ahead of the spermatheca; (3) produce the diverticulum; and (4) bring about the final change in direction of the tubules.

The two processes described above, elongation with shifting of parts, and overgrowth of the dorsal wall, are not independent but overlap in time. This is readily shown by the fact that the folds which form the caudal part of the dorsal elevation begin to form at the "cellular area" stage, and are well developed while the early spermatheca still lies over the vent.

Summary and Conclusions.

1. The cloaca of *Gyrinophilus* females is characterized by the presence of a dorsal elevation which most closely resembles that in *Eurycea*, and a diverticulum like that of the Plethodons.

2. The spermatheca is of the complex type, with a branched common tube, and numerous flask-shaped tubules.

3. Suggestive though inconclusive observations on mating and ovulation have been previously summarized (p. 264).

4. Preceded by the appearance of a cellular area, suggesting the invasion of epithelial cords into overlying tissue, the spermatheca begins to develop at the time of metamorphosis, as a mass of tubules, aggregated about a small depression in the dorsal cloacal wall which becomes the common tube.

5. Coincident with a cephalic shift in position of the whole organ, the tubules begin to change their direction to a predominantly caudal one, and the two folds forming the dorsal elevation, develop. This is followed by a fusion of these two folds cephalic to the common tube, elongation and change of form of the latter, completion of the change of direction of the tubules, and finally the development of the diverticulum. These changes in the cloaca are interpreted as being caused by cephalad growth of the dorsal wall at a more rapid rate than that of the ventral wall, and an overgrowth of the whole dorsal wall.

6. Ventral glands alone are functional in the adult, though at the time of their earliest appearance a similar group of dorsal glands appears, caudal to the anlage of the spermatheca. These undergo a limited development, and then atrophy. Remnants of the group appear in most adults.

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EXPLANATION OF FIGURES.

Plate I.

Figs. 1 to 10.—Diagrammatic cross-sections of the cloaca of a typical adult *Gyrinophilus* female of 16.5 cm. The levels at which these were taken are indicated on Fig. 11, a semi-diagrammatic median sagittal section of an animal of the same size. (Magnification, approximately × 11.)

Fig. 1. Section passing through vent and entrance to dorsal diverticulum.

Fig. 2. Through vent and diverticulum.

Fig. 3. Ahead of vent, through diverticulum.

Fig. 4. Ahead of vent and diverticulum; cuts through most caudal part of gland region. Though most of the glands lie slightly more cephalad, their ducts reach the surface at this level, opening upon, and between, the folds of the lateral and ventral walls of the cloaca. Dorsally, the elevation divided into two folds by the median dorsal slit, is shown.

Fig. 5. Through caudal part of spermatheca, showing the two limbs of the dorsal elevation in the dorsal wall of the cloaca; and, above, the two branches of the common tube, with tubules streaming from them.

Fig. 6. Through cephalic part of spermatheca; showing common tube opening into mid-dorsal slit.

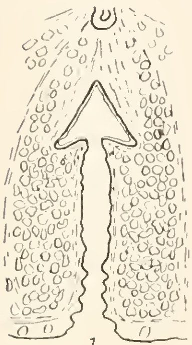
Fig. 7. Cephalic to spermatheca; showing fusion of the two limbs into a single broad dorsal elevation.

Fig. 8. Cephalic to Fig. 7; the dorsal elevation breaking into three folds, of which the median is slender and taller than the other two.

Fig. 9. Through caudal end of bladder; in dorsal wall of cloaca, the three folds seen in Fig. 8; the lateral folds are merging into the lateral wall of the cloaca.

Fig. 10. Through the dorso-ventral expansion of the cloaca. Below the cloaca communicates with the bladder. Into the dorsal expansion, farther cephalad, open the ureters and oviducts. Parts of kidneys are shown above.

Fig. 11. Semi-diagrammatic median sagittal section of cloaca of *Gyrinophilus* female of 16.5 cm. The levels of Figs. 1–10 are indicated.



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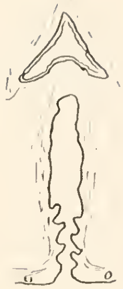

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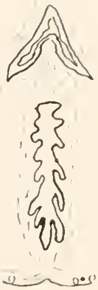

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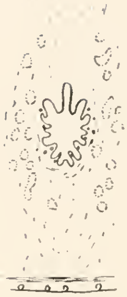

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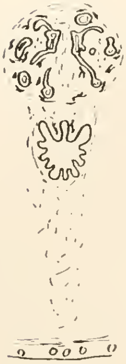

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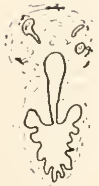

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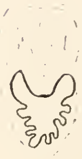

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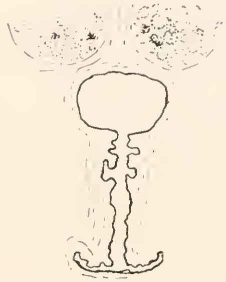

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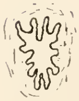

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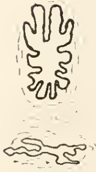

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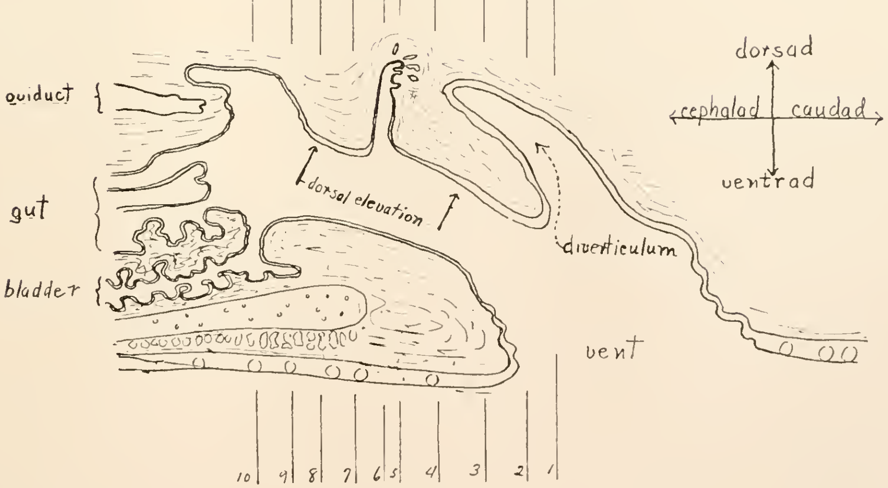
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Plate II.

Fig. 12. Spermatheca at a level mid-way between the levels of Figs. 4 and 5, on Fig. 11. Shows the common tube at its point of junction with its two branches, tubules streaming laterally and ventrally, pigment surrounding tubules, spermatazoa in tubules. (Magnification approximately × 47.)

Fig. 13. Single tubule, showing sperms arranged in whorls. (× 97.)

Fig. 14. Cloaca of female *Gyrinophilus* larva of 8.8 cm., showing median dorsal fold. (Approximately × 33.)

Fig. 15. Same animal; more caudal level than Fig. 14; showing thicker epithelium, with well-defined row of basal nuclei and basement membrane. (× 33.)

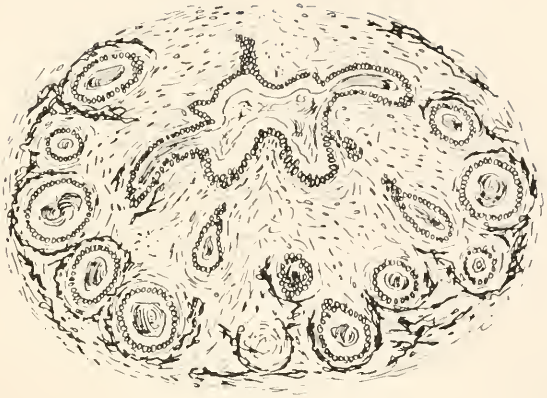
Fig. 16. Cloaca of larva of 10.5 cm., showing cellular area in dorsal wall. (× 33.) *d.e.*, anlage of one fold of the dorsal elevation.

Fig. 16*a*. Portion of cellular area under greater magnification; showing small bulb of cells, the anlage of a spermathecal tubule.

Fig. 17. Cloaca of same animal; showing anlagen of dorsal glands. (× 33.)

Fig. 17*a*. Portion of the dorsal gland area under higher magnification.

Fig. 18. Cloaca of a recently transformed *Gyrinophilus* female of 10.8 cm.; showing caudal end of developing spermatheca. (× 33.) *d.s.*, top of dorsal slit; the pyramidal area above this point is the anlage of caudal portion of common tube, at this stage not yet a tube, but a shallow depression. *d.e.*, one fold of the dorsal elevation.



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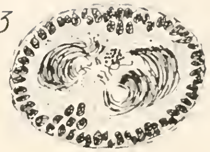

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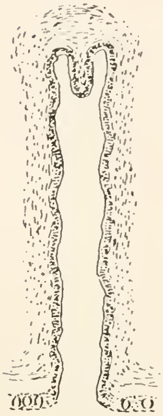

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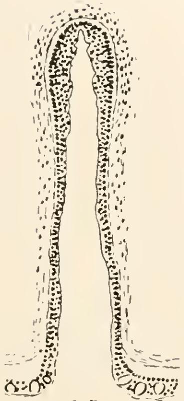

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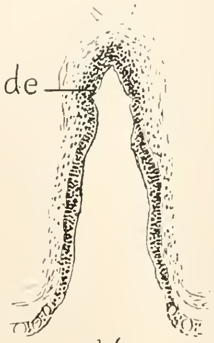

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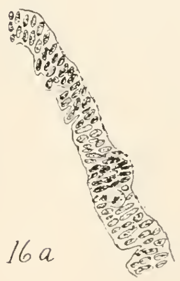

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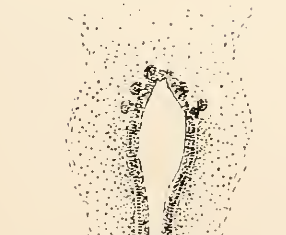

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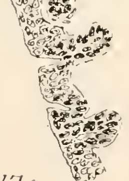

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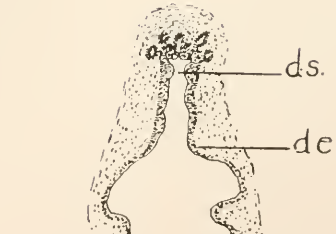

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JOHANNA M. DIECKMANN.

PLATE III.

FIG. 19. From the same animal as Fig. 18, Plate II.; taken at a more cephalic level; showing spermathecal tubules growing out from the area of highly nucleated epithelium. (× 33.) *d.s.*, top of dorsal slit. *d.e.*, one fold of dorsal elevation.

FIG. 20. Dorsal elevation and spermathecal region from an animal of 10.7 cm. (× 33.) *d.s.*, top of dorsal slit. *d.e.*, one fold of dorsal elevation.

FIG. 21. Same animal as Fig. 20; taken at a more cephalic level. (× 33.)

FIG. 22. Dorsal elevation and spermatheca of animal of 11.0 cm.; showing tubules which have been turned latero-ventrad; in the center the narrow necks of tubules which terminate farther caudad. (× 33.) *d.s.*, top of dorsal slit. The cloaca is greatly distended, therefore, the two folds of the dorsal elevation are spread widely apart.

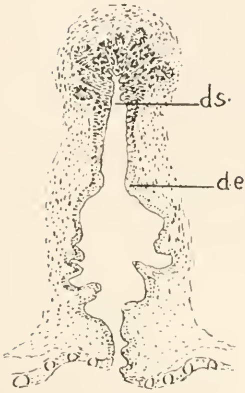
FIG. 23. From the same animal, taken at a more cephalic level; showing the Y-shaped portion of the common tube communicating with the dorsal slit; and irregular lumina forming in the cell mass above the common tube. (× 33.)

FIG. 24. Cloaca and spermatheca of an animal of 11.2 cm.; showing the two limbs of the dorsal elevation in the dorsal cloacal wall; above, the two branches of the common tube, with which tubules communicate. (× 33.)

FIG. 25. Dorsal elevation and spermatheca of the same animal, taken cephalic to the level of Fig. 24; showing the distal part of the common tube, with an H-shaped lumen. (× 33.)

FIG. 26. Dorsal elevation and spermatheca of same animal; taken at a level cephalic to that of Fig. 25; showing expanded proximal end of common tube communicating with dorsal slit. (× 33.) *d.s.*, top of dorsal slit.

FIG. 27. Diagram of median sagittal section of cloaca of young animal; in dotted lines the outline of an adult cloaca, relatively reduced in size. Arrows *a* and *b* indicate unequal growth of dorsal and ventral walls, which results in shifting spermatheca cephalic to vent and turning tubules caudad. Small arrows indicate overgrowth of dorsal wall, which elongates common tube, and produces dorsal elevation and diverticulum.



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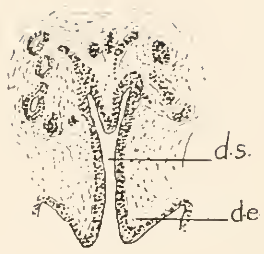

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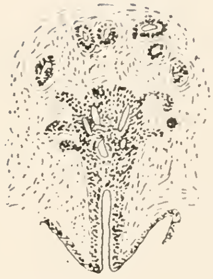

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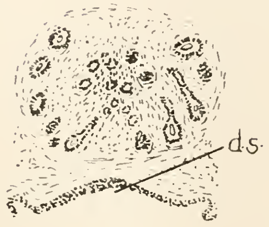

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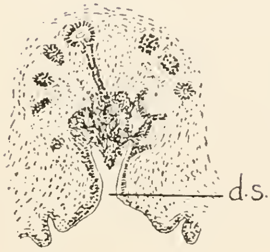

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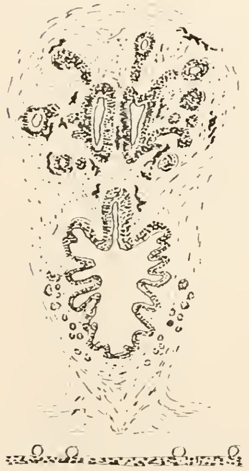

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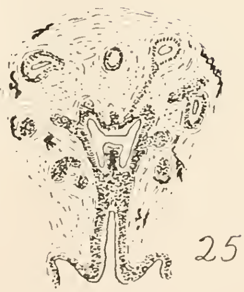

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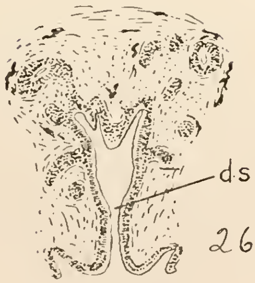

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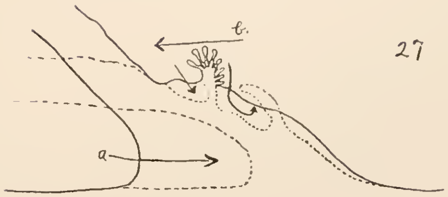

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JOHANNA M. DIECKMANN.

THE CLOACA AND SPERMATHECA OF *HEMIDACTYLIUM SCUTATUM.*

JOHANNA M. DIECKMANN,

Department of Anatomy, School of Medicine, University of Buffalo.

Two specimens of adult female *Hemidactylium scutatum* were examined, one measuring 7.1 and the other 7.7 cm., both captured in August.[1] After fixation in Bouin's fluid, the cloacae were sectioned, one transversely and one sagittally, at 12 μ and 10 μ respectively. The ova of both animals were less than 1.0 mm. in diameter, since ovulation in this species occurs during May (Bishop, '18).

The cloaca of *Hemidactylium,* when empty, is slit-like, with its lateral walls thrown into quite regular folds. Two such folds, located in the dorsal wall of the cloaca, and first discernible at the level of the cephalic end of the vent, become fairly tall as one passes cephalad. These two together constitute the dorsal elevation, which though varying in form, appears in the cloaca of the female in several urodeles (Kingsbury, '95). The slit between the two folds or limbs of the dorsal elevation is continuous caudally with the roof of the cloaca over the region of the vent, and cephalad, with a dorsal expansion of the cloaca, into which, as in other forms, the ureters and oviducts open. In other words, the two limbs of the elevation are not fused in their cephalic portion as they are in adult *Gyrinophilus,* but remain separate throughout their extent, as in developmental stages of the latter. A single median dorsal fold, partially dividing the dorsal expansion, recalls the similar slender fold ahead of and continuous with the fused portion of the elevation in *Gyrinophilus.*

The oviducts have large lumina and folded walls, as is to be expected from the relatively large size of the ova in this form. The bladder opens into the cloaca on its ventral side, somewhat cephalic to the level of the oviducts. There is no dorsal diverticulum (recess), a feature which appears, among the forms so far described, only in the Plethodons and *Gyrinophilus*.

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[1] I am indebted to Dr. R. R. Humphrey for the material used in this study.

The sperm-storing organ is confined to the region above the dorsal elevation. It consists of a few tubules (three on one side and four on the other in one of the animals), which open into the lateral walls of the dorsal slit in its extreme cephalic portion, just at the point where it passes over into the dorsal expansion. From these openings, the long slender necks of the tubules extend caudally; each neck terminates in an irregularly branched end-piece. Branching of spermathecal tubules, observed in only a few instances in the series of *Gyrinophilus,* is conspicuous in the end-pieces of the tubules of *Hemidactylium*. Moreover, some of the terminal branches in this species are not bulbous, but longer and more slender, resembling large tubular glands. The region in which the spermathecal tubules open is free from glands, but opening into the more caudal portion of the dorsal slit, intermingled with the caudal ends of the spermathecal tubules, there is a group of seven or eight pairs of very small, slightly tortuous gland tubules.

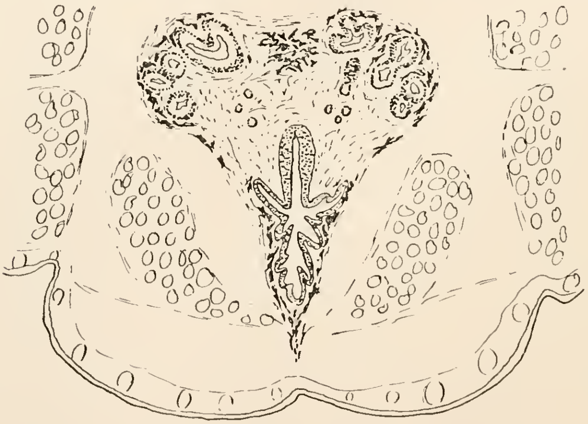

FIG. 1. Cloaca of *Hemidactylium female* (adult, 7.1 cm.), at a level some distance caudal to the openings of the spermathecal tubules. In the dorsal wall of the cloaca, the two folds comprising the dorsal elevation are seen, with the median dorsal slit between them. Just above, on each side, are the narrow necks of spermathecal tubules, which open separately into the dorsal slit cephalad to this level; above these, the expanded, branched end-pieces of spermathecal tubules. Pigment is seen between the two groups of tubules, around the organ as a whole, and in the wall of the cloaca. (× 30.)

The glands described here are all confined to the region of (*i.e.*, located in the substance of) the dorsal elevation, but there are in addition two other groups. One group, consisting of four or five tubules on each side, lying dorsal to and opening into the roof of the cloaca, is located entirely caudal to the elevation. This group is separated by a short but definite interval from those intermingled with the spermathecal tubules. The other group, also of four or five pairs, is located in the region of the dorsal expansion, therefore cephalic to the spermatheca.[2]

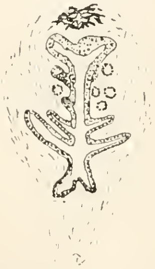

FIG. 2. Cloaca of *Hemidactylium* (adult, 7.1 cm.) at a level through the openings of the spermathecal tubules into the lateral walls of the mid-dorsal slit. Comparison with Fig. 1 shows that, as the tubules pass cephalicly toward their openings, they also slope ventrally, and now lie within the substance of the dorsal elevation. The elevation is composed of two folds or limbs with the mid-dorsal slit between them. The elevation ends abruptly a few sections cephalic to this level. Above, the mid-dorsal slit is already merging into the dorsal expansion ahead of the elevation. The tubules on the right side end one section cephalic to this level; on the left side, two of the three tubules are seen bending sharply toward the slit. (The difference between the sides is due to slight obliquity of sectioning.) Pigment appears above the cloaca. (× 30.)

The epithelium of these glands is emphatically not that of functional gland tubules, as seen in the ventral group in adult *Gyrinophilus;* for the tubules are minute, the lumen barely discernible in most of the tubules, and the cytoplasm in each cell scanty. They closely resemble early stages in the development of glands in young Gyrinophilus females, and are apparently rudimentary or only slightly developed structures. Rudimentary dorsal glands were found in *Eurycea* (*Spelerpes*) in two groups (Kingsbury '95), one cephalic to and one caudal to the opening of the spermatheca, and in *Gyrinophilus* in one group, caudal to the spermatheca. *Eurycea* and *Gyrinophilus* however, have functional ventral glands, while *Hemidactylium* has none, in this respect resembling the Plethodons and *Desmognathus*.

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[2] Another adult measuring 5.5 cm. (with ova 1.0 mm. in August), which has since been examined, shows only the most cephalic of these three groups of glands. They are probably quite variable, as are the rudimentary glands of *Gyrinophilus.*

Bishop states that mating in *Hemidactylium* probably takes place in the spring; he bases this conclusion on the fact that males and females may be found together beneath logs or stones at this time of the year, and on the observation that the ova in the ovaries of animals in the succeeding month, were all less than 0.5 mm. in diameter. The latter fact, to my mind, indicates only that ovulation has recently occurred, and proves nothing as to the time of mating. In the present study, the spermathecal tubules of one of the animals (7.7 cm.) killed in August, are filled with sperms. These are arranged in whorls, as has been noted in other species when sperms are abundant. The animal would seem to have mated after its May ovulation (ova in the ovary are less than 1.0 mm. in size). This finding is in harmony with the idea that sperms may be stored in the spermatheca of urodeles for a considerable time, and that the organ is a device for insuring fertilization of ova in animals which lead a solitary life for a large part of the year.

Gyrinophilus and the seven forms described by Kingsbury, fall rather naturally into a phylogenetic series, as was discussed in the previous paper. The trend during phylogeny seems to have been toward the aggregation of the tubules about a specially modified portion of the cloaca [3] the reduction in number of tubules and loss of their flask shape, and the suppression of glands. *Hemidactylium* cannot be placed in the series, which begins with *Amblystoma,* and which is characterized by the modification of a portion of the cloaca into a common tube, for it has retained the primitive plan of spermatheca seen in *Necturus;* namely, tubules opening separately on the lateral walls of a mid-dorsal slit. It typifies nevertheless, two other tendencies noted in the phylogenetic series; namely, reduction in number and change in form of spermathecal tubules, and reduction in number and functional activity of glands.

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[3] Kingsbury's suggestion that the depression into which spermathecal tubules open in *Amblystoma*, and the common tube of the organ in more highly developed forms, are really a portion of the cloaca, seems justified by the manner of development of the spermatheca in *Gyrinophilus*, and in *Eurycea* (Koehring, '25).

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THE STRUCTURE AND TRANSFORMATION OF THE LABIUM OF *ANAX JUNIUS*.

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Introduction.

During a recent investigation of the degenerative changes in the muscles of transforming Odonata the author found it convenient to use the retraction of the labium as an index to approaching internal changes. For some hours before the final ecdysis the insect remains quiet, without feeding and partly out of water. It is during this time that the retraction of the labium becomes evident. Some attention was consequently given to its condition and structure. When the literature of the subject was searched it yielded little upon structure, beyond that of the chitinous skeleton, and nothing regarding changes during transformation, in spite of the fact that the larval labium has long been cited in both popular and technical treatments as one of the most uniquely adaptive structures in insects. To quote Amans, " Cette piece a de tout temps été jugée comme très curieuse et très intéressante ; tous l'ont décrite avec une minutieuse complaisance, et Dufour en profite pour faire intervenir la Providence et les causes finales."

Miss Butler's paper (2) is quite universally cited as the authoritative treatment of the Odonate labium. It is based upon extensive comparative studies of the configuration of the labium in all of the subfamilies, and her bibliography includes the works of many of the greater insect anatomists as far back as 1842. Rather unaccountably, however, she omits Amans' paper on the larva of *Aeschna grandis* (1), and states that " The powerful muscles and hinges which constitute the mechanism of the labium have never hitherto been investigated " (p. 112). This is followed by a very brief and apparently incorrect description of the mechanism. Her interpretations are in turn followed by Tillyard (4) in 1917.

Amans (1) studied the larval labium with considerable care but he, also, overlooked some of the most evident and important structures, and was consequently led astray regarding most of the major functions, *i.e.*, extension and flexion of the labium and the abduction of the lateral lobes. It may further be stated that he applied no modern system of nomenclature to the muscles and other structures described.

Neither of these authors published adequate figures of the larval structures. All available illustrations of the imaginal labium are concerned solely with the skeletal parts, and the transitional stage has never been figured.

For these reasons an investigation of the structure of the labium of the larva and of the adult and its transitional condition has seemed worth while.

Materials and Methods.

The specimens of *Anax junius* studied were fixed in Bouin's fluid or injected with 95 per cent. alcohol. Both fluids gave good results, though the yellowing of the muscles by the picric acid made it possible to clear and mount dissections without resorting to further staining. In fact, the definition and relations of the muscles in such preparations was often superior to those stained in borax carmine. Specimens were fixed in both the extended and flexed positions.

While a part of the work was done from cleared and mounted specimens, details of the structure were obtained much more efficiently by the dissection of alcoholic materials under a Greenough binocular microscope. By this method the parts could be pinned down in 70 per cent. alcohol in a translucent paraffin tray for dissection with needles, and viewed alternately by reflected and transmitted light. Care was taken to clear away all fat or coagulated body fluids which might interfere with clear vision. Adult labia were cleared and studied in oil without mounting.

Most of the drawings were outlined under the camera lucida, though a few were made to measurement.

The Labium of the Larva.

General Structure.

The labium of a full grown larva is between sixteen and twenty millimeters long when extended (Fig. 1). It consists of two main divisions, the submentum and the mentum. The latter is somewhat the longer of the two, and is roughly spatulate in shape; the former is subcylindrical.

The mentum terminates apically in a rounded, median lobe which bears a median notch and is edged with short bristles. On either side of the median lobe is a lateral lobe bearing a moveable hook and an end hook. These possess no muscles. Each lateral lobe is hinged near the outer border of its base, while the medial lobe is prolonged into a long triangular tendon which lies in a deep slit-like invagination in the anterior edge of the mentum and receives the insertion of the adductor muscle. Proximally the mentum narrows to a trifle more than half its apical width, and deepens to accomodate the heavy adductor and abductor muscles. It is hinged to the submentum as shown in the various figures, the carinae being heavily chitinized.

The submentum is flattened on the face opposed to the mentum, otherwise it is nearly cylindrical. Its distal end is rounded and projects beyond the hinge at which the mentum is received. It is within this rounded, elbow-like projection (menton, of Amans) that many of the important muscle attachments occur. The submentum is hinged closely to the ventral side of the head but possesses such freedom of movement as to enable it to swing instantly from its normal position between the first and second pairs of legs, when the labium is folded, to one in nearly the same plane beneath and in front of the head. It is supported by the posterior branches of the tentorium and surrounding skeletal parts. A large outward fold between the head and base of the submentum, supported by a T-shaped chitinous rod running forward and downward to the base of the hypo-pharynx (ancre, of Amans), aids in giving this freedom for the forwards thrust (Fig. 1). Little lateral movement is possible at this hinge and practically none at that between the submentum and mentum. The submentum accommodates two pairs each of extensor and flexor muscles.

Larval Musculature.

Muscles Activating the Lateral Lobes.—The lateral lobes are moved by two pairs of strong muscles, the abductors and the adductors.

The abductors of the lateral lobes lie wholly within the mentum (Figs. 1, 10 and 11). They are strong flattened muscles lying laterally, broad at the origin upon the dorsal surface of the skeleton slightly distad from the hinge and tapering to a point at the insertion of the tendon upon the outer side of the lateral lobe, where a slight prominence gives leverage. The mesial side of each abductor overlaps the corresponding adductor muscle. A tracheal branch lies ventral to it laterad of the adductor.

The adductors of the lateral lobes are the largest muscles of the labium (Figs. 1, 8 to 13). The origin is upon the central area of the rounded extension of the submentum where it gains excellent support from the heavily chitinized and concave surface. The muscle tapers to its insertion upon the long tendon attached to the mesial side of the lateral lobe. As shown in Fig. 8 this muscle seems to be comprised of a dorsal and a ventral layer. In Fig. 9 most of the muscle had been dissected off. In the region of the hinge and about their origins the adductors are flanked by the secondary (accessory) flexors (Fig. 13). Fig. 9 shows how the adductors may also aid greatly in flexing the labium. The first effect of their contraction being the adduction of the lateral lobes, that of continued contraction across the hinge, flexion.

Muscles Activating the Mentum and the Submentum.—Four pairs of muscles within the labium are concerned with its extension and flexion, the primary flexors, the secondary (accessory) flexors, the primary extensors, and the secondary (accessory) extensors.

The primary flexor muscles lie in the ventral portion of the submentum (when the labium is folded). They have their origins side by side upon a V-shaped tongue of chitin on the median ventral region of the base of the submentum (Fig. 1). They run distally, accompanied by a pair of tracheal trunks, taper gradually, pass over the hinge lateral to the adductors, and insert upon the ventral surface of the mentum at its proximal end. Each is attached to a short, transverse, chitinized, thickening projecting mesiad from the lateral carina (Figs. 1, 9, 10 and 12). In passing around the adductor muscle it narrows to nearly tendon-like proportions. A comparison of Figs. 8, 9, and 10 will make clear its action as a flexor.

The secondary flexor muscles are located in the region of the hinge. They have not been described previously. In the contracted condition they are somewhat fan-shaped (Figs. 9 and 11). The origins of the muscles lie at their broad ends, the attachments to the submentum nearly circling those of the adductors (Fig. 13). From here they pass ventrally next to the skeleton to insert for a very short distance on the ventro-lateral floor of the mentum just proxinad to the origins of the abductors of the lateral lobes. When the labium is extended they assume so different a form that they have probably been mistaken by earlier workers for the proximal ends of the abductors. The dissection shown in Fig. 10 indicates the true relations. It will be noted that each passes laterad to the chitinous process, which serves for the insertion of the primary extensor muscle.

The primary extensor muscles occupy the dorsal portion, when the labium is folded, of the submentum (Figs. 1 and 8 to 13). Their origins are quite far apart upon the ends of the posterior branches of the tentorium. They are heavy at the bases but each soon tapers to attach to a small cord-like tendon which passes between the adductor of the lateral lobe and the secondary flexor to a triangular chitinous process projecting from the mentum into the cavity of the rounded elbow of the submentum. This mechanism is a very nice adaptation for increasing the leverage of this strong extensor muscle. A comparison of Figs. 9 and 10 leaves no doubt of the mode by which the extensor functions.

The secondary (accessory) extensor muscles have also escaped description in the past. They are small band-like muscles attached to the meso-lateral regions of the elbow of the submentum (Figs. 5, 6, and 8 to 10). Their origins are at the extreme distal end of the submentum; their insertions latero-ventral to those of the secondary flexors. Their function seems to be that of producing in the skeleton of the elbow of the submentum the necessary increased curvature during extension of the labium.

THE LABIUM DURING TRANSFORMATION.

As indicated earlier, a period precedes transformation during which the larva ceases to feed and wanders restlessly in and out of the water, finally remaining quietly on a twig or other support with the thorax in the air. It seems to be during this restless period that the labium is remodeled and retracted into the submental portion of the old skeleton, though it has been impossible to observe the steps in this process. It is accomplished in a few hours at most, perhaps in a much shorter time, as there appears little evidence of profound histological change. Practically all of the stages collected were similar to those illustrated in Figs. 2 and 3. The mentum and lateral lobes of the larval skeleton are entirely empty and the labium has retracted into the basal two thirds of the old submentum. Its parts are straightened out and rolled laterally to fit the tube-like case, while the whole labium stands out ventrally from the under side of the head. When viewed under low magnification most of the parts of the adult labium are visible but they are thicker and more fleshy in appearance and are closely rolled together. The broad lateral and median lobes are especially conspicuous. Attempts to flatten out the excised organ are not usually successful as the tissues are very delicate and the parts tear badly. However, a median longitudinal section dividing the labium into halves, made possible the determination of the following facts.

As all of the internal structures are so crowded together it is difficult to make out their relations. However, the larger muscles are quite clear as to identity. The primary flexor muscles are very long and may be followed laterally around the bases of the adductors as in the larva. Each is accompanied by a large, degenerating trachea. The adductors have contracted to form very short, thick muscles. They are curved ventrally at their origins and insert as in the larva. They can be identified with assurance by their insertion and relations to the primary flexors. The remaining muscles cannot be so definitely followed but may be seen in general outline, tending towards vertical or slightly oblique positions in the mentum and submentum. As these latter are so rolled up, however, it is probable that the muscles might assume diverging positions as in the adult, if the labium was flattened out.

In the ventral, distal portion of the mentum are two rounded masses of some size which suggest degenerating muscles. Comparison of the adult with the larva indicates the loss of the abductors and it is possible that these two masses represent them at this stage. No histological examination has as yet been made.

The tracheæ which are to remain in the adult are seen wound vertically up and down between the main muscles. The T-shaped chitinous support mentioned in connection with the larval labium is very conspicuous, and bears a large flattened tooth-like ventral process.

The Labium of the Adult.

General Structure.

The labium of the adult is much shorter, thinner, and more expanded than that of the larva. When flattened out it measures approximately five millimeters in length by eight millimeters in diameter, including the lateral lobes. That is, it is less than a third as long as, and only a third wider than, the larval structure from which it arose. The added width results from the expansion of the lateral lobes. The latter with the greatly enlarged median lobe, form the mask which covers the mouthparts. In their normal positions the lateral lobes and the median lobe rise almost vertically from the borders of the floor-like mentum-submentum to enclose the sides and front of the mouth; the whole structure is nearly cup-like.

The function of the labium is changed to a considerable extent. In the larva it is a remarkable organ of prehension, in the imago it has become mainly one for the retention of prey caught in the basket of the spiny legs. Its moveable hooks now resemble palps possessed of a single small terminal tooth or spine each. While formidable in size and appearance the end hooks are weakly joined to the lobes (Fig. 4). They would be of little use without the pressure and support applied to their outer faces by the flexing of the median lobe (Fig. 7), converting them into a sort of fork to hold the food in the mouth.

The mentum is relatively short and very wide due to the formation of a pair of squames laterally by the development of longitudinal folds and chitinous ridges along the areas of muscle attachment. The hinge between it and the submentum is less flexible than in the larva, but possesses a distinct enlargement along its median half. Most of the muscle attachments are grouped on either side of this enlarged portion. The submentum is greatly reduced but offers support for the origin of all of the larger muscles (Figs. 4 to 7). The unique adaptations for extension and flexion so marked in the larva are gone.

Adult Musculature.

There are five pairs of muscles in the imaginal labium. These are greatly reduced in size and lack much of the precision of adjustment of the earlier condition. A comparison of the larval, transitional, and adult structures has led to the following homology. There is nothing to indicate that more than one pair has been destroyed. Many are clearly identified by their attachments and relations, while a few others have been difficult to determine except by elimination and by evident function. They may be located on Figs. 4 to 7.

Muscles Activating the Lateral Lobes.—The abductors of the lateral lobes. These muscles are missing in the imago. The two rounded masses of apparently degenerating muscular tissue in the transitional labium may represent them. Abduction of the lateral lobes is now accomplished indirectly by the pull of the secondary flexors on the squames.

The adductors of the lateral lobes. This pair of muscles is clearly in its larval position. The origin of each is upon the ventral wall of the submentum just proximal to the hinge or angle. The area of attachment is circular or oval in form, and is close to the median line. The insertion is, as in the larva, upon a strong tendon at the inner angle of the lateral lobe. This inner angle is distinctly offset dorsally (*i.e.*, towards the buccal cavity) so that a contraction of the muscle produces flexion of both the lateral lobe and the median lobe as well as adduction of the former. In shape it is subcylindrical and tapers from its origin to its insertion in a half spindle. It lies mesiad of other muscles and somewhat parallel to the primary flexor.

Muscles Activating the Mentum and Submentum.—The primary extensor muscles are the longest in the adult labium. They are of depressed spindle shape, and lie in the median line between the bases of all the diverging muscles. In dissecting the labium free from the head they are often torn loose at their insertions and withdrawn. The origins are upon a chitinous semi-circle near the base of the submentum; the insertions on the dorsal surface (*i.e.,* towards the buccal cavity) of the extreme proximal region of the mentum, one immediately on either side of a slight median carina-like thickening.

The secondary (accessory) extensor muscles. These small muscles lie transversely on the floor of the apex of the submentum, one on either side ventral to the middle region of the primary and secondary flexors. They are of a depressed cone shape, about 40° in diameter at the origin. The origin is upon a chitinous semi-circle projecting from the ventral wall of the distal end of the submentum midway between the median line and the lateral margin. The insertion is upon a short tendon from the extreme proximo-lateral angle of the squame. Without careful focusing with a 16 mm. objective this seems on the lateral margin of the submentum; practically, the pull is upon both at once.

The primary flexor muscles are probably the strongest pair in the labium. They are heavy and cylindrical, and lie between the primary extensors and the adductors medially and the secondary flexors laterally, dorsal to the transverse secondary extensors. They originate on semi-circles at the base of the submentum with their inner sides close together. The insertion of each is on a fairly large circular area of the ventral surface of the mentum at its proximo-lateral angle (exclusive of the squame), and marked on the surface by a convexity lateral to the thickened central portion of the hinge.

The secondary flexor muscles are thin and band-like, and twisted at an angle of about 90° about the lateral aspects of the primary flexors. They are crossed midway and ventrally, as indicated above, by the secondary extensors. The origin is upon a transverse chitinous semi-circle located ventro-laterally about midway of the length of the submentum. The insertion is upon a similar semi-circle on the latero-dorsal aspect of the squame.

Summary.

Studies carried out upon the larval, transitional, and adult stages of development show that:

1. But one pair of muscles breaks down (the abductors of the lateral lobes) during transformation. The remainder are reduced in size and changed in their attachments only in so far as the new functional conditions necessitate.

2. Changes in the function of certain pairs is clear, as in the secondary flexors which take on the duty of abduction in the imago.

3. Two pairs of muscles are here described for the first time: the secondary extensor, and the secondary flexor muscles. New details regarding the mechanism of extension and flexion are also described.

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DESCRIPTION OF FIGURES.

All figures are of the labia of *Anax junius*. The drawings were made through the camera lucida or to measurement by the author. Dotted lines indicate the forms of parts which underlie others and which could usually not be seen without further dissection or the clearing of the specimen. The magnifications are expressed in diameters.

adl —adductor muscle of the lateral lobe.
abl —abductor muscle of the lateral lobe.
bc —buccal cavity, mouth parts removed.
ca —carina.
exp—primary extensor muscle.
eh —end hook.
exs —secondary or accessory extensor muscle.
fxp—primary flexor muscle.
fxs —secondary or accessory flexor muscle.
hb —hind border of the head.
hy —hypo-pharynx.
hd —head, sectioned.
kr —chitinous T-shaped rod.

lal —lateral lobe.
m —mentum.
ml —median lobe.
mh —movable hook of lateral lobe.
mx —base of maxilla.
sd —opening of salivary duct.
skl —skeleton of larval labium, severed at hinge between the mentum and submentum.
sm —submentum.
sq —squame of mentum.
tn —central plate of tentorium.
tnp —posterior branch of tentorium.
tr —trachea.

PLATE I.

Fig. 1. Extended labium of the larva, viewed from below and showing the muscles and tendons as seen through the transparent skeleton. × 6.

FIG. 2. Retracted labium of the larva as it appears a short time before the insect transforms. Ventral view of head and dorsal view of the labium. The hypo-pharynx, bases of the maxillæ and other structures are shown surrounded by the loose larval skeleton. × 9.

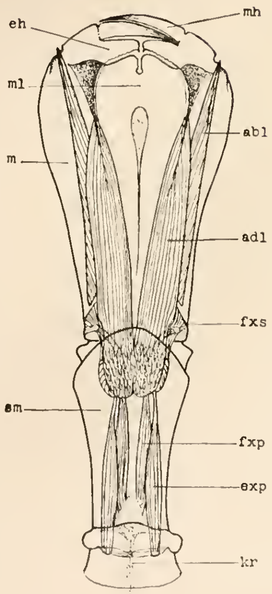
FIG. 3. A ventral view of the same labium shown in Fig. 2. × 9.

FIG. 4. Extended and flattened labium of the adult in ventral view showing the muscles. × 9.

FIG. 5. Ventral view of the mentum of the adult. The labium in this and the next two figures is in normal condition, not flattened out. The muscles and their attachments are drawn in one half only in order to show the skeletal features on the opposite side. × 9.

FIG. 6. Ventral view of the submentum of the specimen shown in Fig. 5. Other conditions the same. × 9.

FIG. 7. A view into the cup-like labium of the adult from the dorsal aspect. The muscle relations are shown, and in addition the mechanism of the end hooks and the median lobe. × 9.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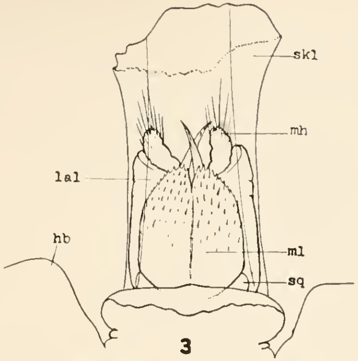
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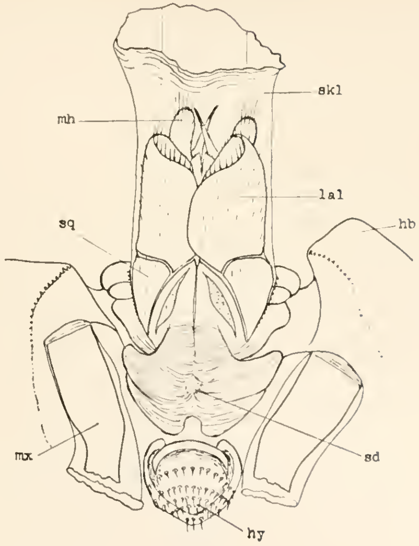
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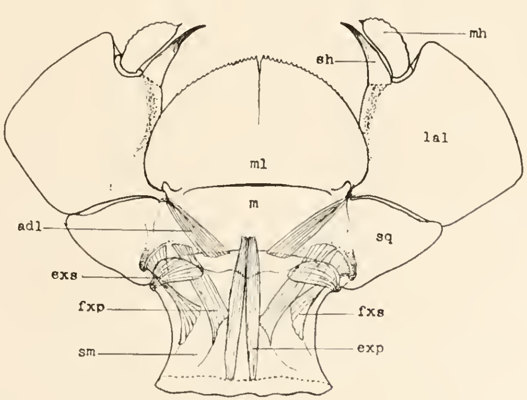
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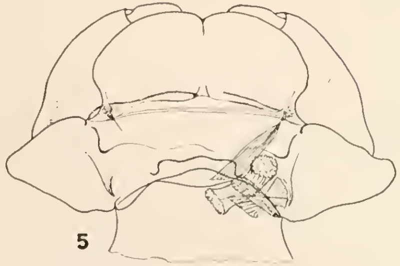

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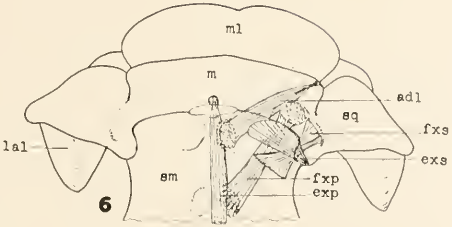
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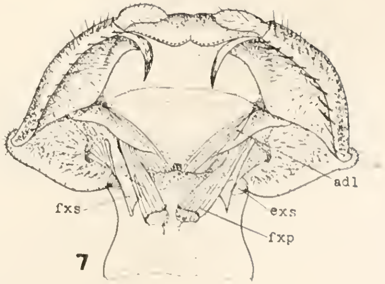
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PLATE II.

FIG. 8. Right half of the head and flexed labium of the larva as viewed from the inner aspect. Parts of the tracheal branches which lie between the adductor muscles are omitted. × 9.

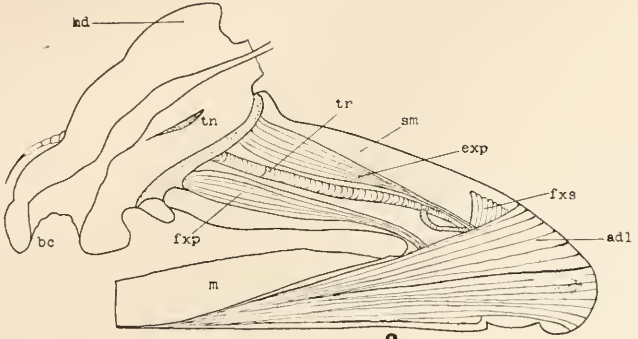
FIG. 9. Left half of the labium shown in Fig. 8, so placed as to illustrate, together with Fig. 8, the complete labium opened like a book. In Fig. 9 the adductor muscle has been dissected away to give a complete view of the mechanism for extension and flexion. Chitinized processes are stippled. × 9.

FIG. 10. Left half of the extended larval labium as seen from the interior. A large portion of the base of the adductor muscle is dissected away to uncover the extensor and flexor muscles. The primary and secondary flexors should be especially noted. × 9.

FIG. 11. The flexed larval labium viewed from the exterior right side, the muscles showing through the transparent skeleton. × 9.

FIG. 12. Ventral view of the mentum and the main hinge of the larval labium when flexed. All internal parts seen through the skeleton. × 9.

FIG. 13. Dorsal view (with labium flexed, ventral when extended) of the submentum of the specimen shown in Fig. 12, illustrating the attachments of muscles. × 9.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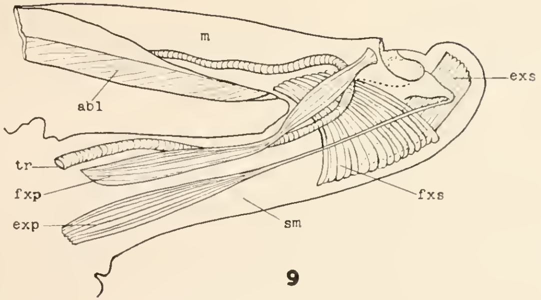
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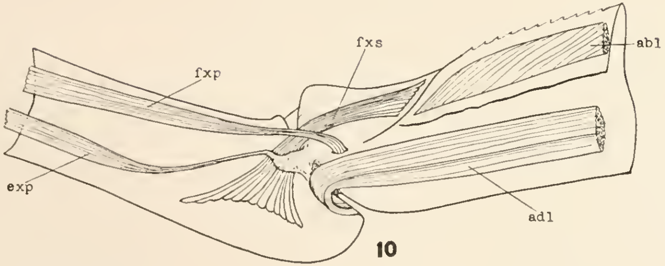
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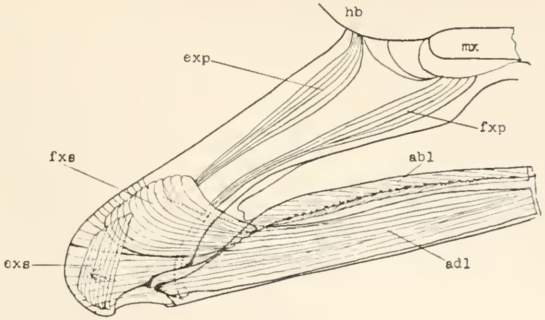
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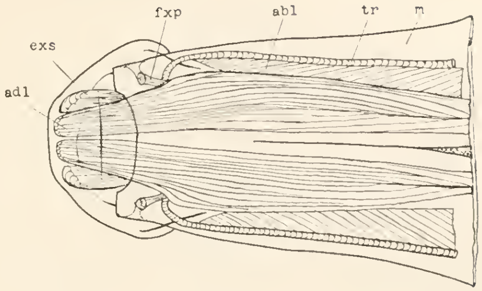
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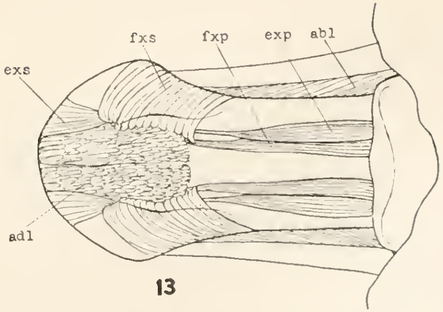
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BIOLOGICAL BULLETIN

STUDIES IN ANIMAL AGGREGATIONS: THE RELATION BETWEEN MASS OF ANIMALS AND RESISTANCE TO COLLOIDAL SILVER.

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One of us has been interested for many years in studying the causes and effects, particularly the physiological effects, of close aggregation upon animals. Much of the literature dealing with this problem has been surveyed elsewhere (Allee, 1927). The work on which the present paper is based was stimulated by the repeated reports of Drzewina and Bohn (1921–1926) of which the general results can best be summarized in their own words (1926):

"Nous avons recherché l'intervention du facteur *masse* dans les réponses de divers organismes vis-à-vis de multiples agents nocifs du milieu extérieur. Dans une masse M d'eau, on introduit une masse *m* d'un être vivant (*m* étant égal ou inferieur à M/100), cette masse résiste à un agent nocif déterminé (substance chimique par exemple) ; mais une masse plus petite, *m*/10 ou *m*/100, ne résiste pas; tout se passe comme si la masse de matière vivante exerçait, vis-à-vis d'elle-même, un effet protecteur (auto-protection)."

Drzewina and Bohn worked much with colloids of the heavy metals, particularly with colloidal silver. Accordingly we have used the same reagent, made as follows, from directions furnished by Dr. Terry-McCoy of the Department of Chemistry of the University of Chicago: "Dissolve 4 gm. of commercial dextrine in

100 cc. of water and then 4 gm. of pure caustic soda. Dissolve 3 gm. of silver nitrate in 20 cc. of water and add to the dextrine-soda solution. The precipitate of silver oxide that is formed is gradually reduced by the dextrine, the color changing to reddish brown. After 20–30 minutes, add 100 cc. of 90 per cent. alcohol and stir. Allow the mixture to settle for 15–20 minutes and then pour off the liquid from the particles of silver at the bottom. Add about 200 cc. of water and the silver particles will generally disperse; however, if this is not the case shake or stir until an even suspension of silver results."

With dilutions from such stock suspensions, it was easy to demonstrate, as Drzewina and Bohn state, that within limits, the greater the mass of animals, the better the protection. Thus with *Planaria dorotocephala,* 12 sets of two each exposed at room temperature to 10 cc. of water containing 10 drops of a rather weak suspension of colloidal silver, showed the beginning of head disintegration in from 4.5 to 10 hours; while 10 similar lots each containing from 10 to 75 worms, all lasted over 36 hours in the same volume and concentration. A species of Cladocera and the isopod, *Asellus communis,* showed similar, but less marked group protection.

Similar tests in which the animals were exposed to the action of dilute suspensions of colloidal silver for considerable time and were then washed and transferred to water similar to that to which they were accustomed, yielded such results as are listed in Table I. With the worms, the results are summarized in terms of survival after exposure, with added information concerning the number of worms that were able to crawl either with or without stimulation with a camel's hair brush. Other data at hand show that the protective effect of the group was greater than is indicated by this summary, but the summarized account is sufficiently conclusive. With the brittle starfish, *Ophioderma,* tests were made concerning the righting ability of the animals after exposure to colloidal silver. In these tests, after exposure, the washed animals were transferred to separate finger bowls each containing 250 cc. of well-aërated sea water. It will be noted that the mean righting time of the isolated animals is given in minutes while that of the animals tested in groups of five is given in seconds.

TABLE I.

SHOWING THE RESULTS OF EXPOSURE TO SUSPENSIONS OF COLLOIDAL SILVER FOLLOWED BY TRANSFER TO WATER SIMILAR TO THAT TO WHICH THE ANIMALS WERE ACCUSTOMED.

All were run at room temperature.

Animal.	Cc. of Water.	Drops Colloidal Silver.	Number Animals.	Time Exposed Colloidal Silver.	Survive.	Moving.	Died.
Planaria maculata . . .	15	12	6(3)	2 hrs.	11	2	7
	15	12	1(75)	2 hrs.	72	66	3
	5	1	5(3)	26 hrs.	9	0	6
	5	1	1(25)	26 hrs.	25	23	0
Planaria dorotocephala	10	2	40(1)	7 hrs.	15	1	25
	10	2	3(25)	7 hrs.	75	63	0
Dendrocoelum lacteum	5	1	5(3)	26 hrs.	3	1	12
	5	1	1(24)	26 hrs.	18	18	6
Ophioderma brevispina					Righted	mean time	
	50	2	48(1)	14.5–16 hrs.	41	30 min.	0
	50	2	12(5)	14.5–16 hrs.	59	38 sec.	0

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More cases with different concentrations could be given but the result is the same in all. This agrees entirely with the experience of Drzewina and Bohn, who used a wide range of animals; and with that of Bresslau, using different toxic reagents with ciliates.

A priori one would expect exactly such a result since in each case there is much less of the toxic substance present per individual composing the larger groups, than when 1–3 animals are placed in the same amount of the same concentration of toxic substance (Pieron, 1921). Yet the possible menace of crowding, so frequently given as the only result of confining many animals within a small space, may easily be demonstrated even here. With the *Ophioderma,* for example, if the glass fingerbowls were covered with glass plates during the exposure to colloidal silver, thus stopping free gaseous exchange, the grouped animals were in much worse condition than were their isolated fellows.

CAUSE OF GROUP IMMUNITY.

When one begins to inquire into the causes of the greater immunity of the grouped individuals, he immediately finds division of opinion. Drzewina and Bohn summarize their conclusions repeatedly in some such words as the following (1921*d*): "Bref, si chez diverses espèces que nous avons examinées jusqu'ici (Paramécies, Colpodes, Stylonychies, Stentors, Hydres, Convoluta, Glossiphonies, têtrads de Grenouille) tout se passe comme s'il y avait émission rapide d'une substance ou de substances assurant une défense; chez *Polycelis,* c'est le contraire (against KCl): on assiste, non pas à une *auto-protection* mais à une *auto-destruction.*"

They do not believe that the greater resistance of the group is due to a more rapid using up of the toxic substance, and cite two types of experiments to support their contention (1921). In the first type they expose some hundred (centaine) of Convoluta in one suspension and two each in the accompanying dishes. Even if the former receive 10 drops of colloidal silver to 25 cc. of water and the isolated animals have only two drops for the same volume, they find that the large group survives after the others are disintegrated. This is a fairer test than if the same amount of colloidal silver were added in both cases, but still it must be remarked that the ratio of colloidal silver per individual is about 10 times greater in the case of the two individuals, when compared with the larger group.

We have made some exact tests to cover this point and present the following summary of our results:

Animals Used: Planaria dorotocephala, 15–25 mm. long, exposed to action of colloidal silver for 7 hrs., then washed and transferred to water to which they were accustomed and examined on the day following the exposure.

I. 40 planarians isolated into 10 cc. of water plus 2 drops colloidal silver.

	No.	%
Normal, crawling when stimulated	1	2.5
Less than half disintegrated	2	5.0
Half and less than two thirds disintegrated	4	10.0
Two thirds but not all disintegrated	8	20.0
Wholly disintegrated	25	62.5

II. 150 planarians in 6 lots each in 250 cc. water plus 50 drops colloidal silver.

	No.	%
Normal, crawling when stimulated	0	0
Less than half disintegrated	0	0
Half, but less than two thirds disintegrated	18	12
Two thirds, but not all disintegrated	52	35
Wholly disintegrated	80	53

III. 71 planarians in three lots each in 10 cc. of water plus two drops of colloidal silver.

Normal, crawling when stimulated	63	88.8
Normal, not crawling when stimulated	1	1.4
Head started to disintegrate	7	9.8
Head completely disintegrated	0	0.0

IV. 75 planarians in three lots each in 10 cc. of water plus 50 drops of colloidal silver.

All wholly disintegrated.

The tests reported show clearly that if a group of 25 Planaria are placed in the same volume and the same concentration of colloidal silver as are isolated individuals, that the former survive the experience in good condition while the majority of the latter succumb, and almost all the others are severely affected. Further, if similar planarians are placed at the same time in the same volume of water, to which has been added sufficient colloidal silver to make the concentration per individual equal to that given the isolated worms, the bunched animals all succumb to a treatment that left 37.5 per cent. of the isolated individuals alive, even if most of them were strongly affected. And finally, if we place the groups in proportionate volume and proportionate concentration of colloidal silver, their condition approaches that of the isolated individuals. We conclude from this evidence that the greater protection which the mass furnished when exposed to colloidal silver is due to the smaller amount of the toxic substance which each individual has to remove from suspension in order to lower the strength below the threshold of immediate toxicity. The mechanism for this removal will be discussed later.

Another type of experiment was run with *Ophioderma* to determine whether or not the protective effect of the bunch was due to the fact that the bunched animals were exposed to a smaller concentration of colloidal silver than were the isolated animals. Since the area of the surface would affect the aëration and so affect survival values, the larger numbers were placed in crystallization dishes with a diameter of 24 cm., while the smaller bunches and the isolated animals were in the usual finger bowls with a diameter of 10 cm. The essentials of the set-up were:

One bunch of ten animals with 25 cc. sea water and 1.5 drops of colloidal silver each, and with a surface exposure of 45 sq. cm. per animal.

One bunch of 5 animals with 5 cc. of sea water and 0.3 drops of colloidal silver each, and with a surface exposure of 15.6 sq. cm. per animal.

Five isolated animals with 25 cc. of sea water and 1.5 drops of colloidal silver each and with 78 sq. cm. surface exposure.

A second set was exactly similar, except that the animals were exposed to 2, 0.4, and 2 drops of colloidal silver per individual.

The exposure in a typical experiment lasted 15.5 hours, after which the animals were washed as usual and transferred to fresh sea water for observation for the following 24 hours. At intervals records of numbers righted after having been turned over, and other evidences of activity, such as spontaneous motion of tube feet, were recorded.

All of the animals bunched in the 10 cm. fingerbowls recovered sufficiently to right themselves within 24 hours, despite the fact that four of the ten were much corroded by the action of silver that had settled to the bottom of one of the fingerbowls. In both experiments, the isolated animals fared next best, although they made a much poorer showing than did the bunched individuals that were exposed to a much lower amount of colloidal silver per animal; only six of this set of ten righted. The bunched animals, exposed to the same volume and amount of colloidal silver as those isolated, ranked a poor third, which may be due to the decreased surface exposure, or to some of the better known ill effects of crowding.

These experiments indicate either that the decreased amount of colloidal silver or the reduced volume of sea water present per individual, or both, markedly favored the survival of the animals bunched in the small amount of water present in the fingerbowls. Drzewina and Bohn emphasize the latter factor, and accordingly the following experiments were run to test out this point.

Lots composed of four solitary *Ophioderma* and one bunch of five were exposed in fingerbowls to 25, 50, 100, and 250 cc. of sea water, each containing only two drops of the stock suspension of colloidal silver. After 15 hours they were washed and tested for activity as usual. Those in 250 cc. of sea water, where the colloidal silver suspension had a value of one drop to 125 cc., righted immediately whether they had been bunched or isolated.

Those in 100 cc., with the colloidal silver ratio of 1 drop to 50 cc. all righted; the bunched animals in from 15 to 30 seconds, mean 27 seconds; the isolated in from 15 seconds to 8 minutes, mean 161 seconds.

The bunched animals in 50 cc., with the colloidal silver ratio of one drop to 25 cc., all righted in from 10 to 220 seconds, mean 101 seconds. Two of the accompanying isolated animals righted, one in 95 seconds and the other after an hour and a half. The others did not right in the next two days.

The bunch in 25 cc., with one drop to 12.5 cc. all righted in from 3 hours, 38 minutes, to about six hours, with a mean of 4 hours, 41 minutes. One of the isolated individuals righted almost immediately but none of the others did so. Contrary to the experience of Drzewina and Bohn (1921*a*), the toxicity depends on the concentration of the colloidal silver, rather than on the volume to which the animals are exposed.

It is worth noting that the protective action of the bunch appears only in the more toxic suspensions, where apparently the toxic strength per individual was more nearly reached even with the bunched animals. Drzewina and Bohn apparently would interpret such an observation as meaning that the auto-protective substance is secreted more rapidly in more toxic conditions.

Reconditioned Solutions.

The second and more convincing experiment used by Drzewina and Bohn as evidence in favor of their view that the group protection is furnished by some sort of auto-protective secretion, rather than by exhaustion of the toxic substance, is as follows (1921*a*):

"Nous décantons la solution où depuis 24 heures séjournent une cinquantaine d'embryons (tadpoles of *Rana fusca*) et dont la teinte révèle la presence du colloïde; nous y ajoutons le même nombre de gouttes que la veille, 1 par exemple, et nous y plaçons deux embryons neufs du même âge. Ceux-ci survivent, alors que des individus témoins, placés dans une solution neuve à 1 goutte de collargol, succombent, comme c'est la règle pour les isolés. Il semble ainsi que, attaquées par le colloïde, les larves émettent, *rapidement,* une substance (ou des substances) qui a pour effet de les protéger."

Wholly similar experiments were run with the brittle starfish, *Ophioderma*, to test for the presence of the postulated auto-protective secretion. Colloidal suspensions in which *Ophioderma* had been exposed were reconditioned by three hours aëration with room air in order that the new lot might not suffer from low oxygen tension, and were filled to the original volume with distilled water. The original suspension had been made with one drop of colloidal silver for each 25 cc., and the same amount was added to this reconditioned water. Tests showed that the aëration of freshly prepared suspensions for this length of time caused a color change but did not markedly affect the toxicity.

Three lots, each consisting of one group of five bunched animals and four isolated individuals, were placed severally in 50 cc. of such a reconditioned suspension, nine hours after the previous experiment had closed. These were run simultaneously with three similar lots in freshly prepared suspensions of two drops of colloidal silver in 50 cc. of sea water. All conditions were similar. The temperature was 18.5°. The results are summarized in Table II.

TABLE II.

SHOWING RIGHTING TIME OF *Ophioderma* AFTER AN EXPOSURE OF 17 HOURS IN RECONDITIONED AND FRESH SUSPENSIONS OF COLLOIDAL SILVER.

All gave the righting reaction.

Reconditioned Suspension.

<table>
<tr><th colspan="3">Bunch.</th><th colspan="3">Isolated.</th></tr>
<tr><th>Number.</th><th>Mean Time.</th><th>Spread.</th><th>Number.</th><th>Mean Time.</th><th>Spread.</th></tr>
<tr><td>15</td><td>73″</td><td>10″–12′</td><td>12</td><td>25″</td><td>5–118″</td></tr>
<tr><td colspan="6"><i>New Suspension.</i></td></tr>
<tr><td>15</td><td>20″</td><td>7–75″</td><td>12</td><td>25″</td><td>10–65″</td></tr>
</table>

If Drzewina and Bohn were correct in thinking that the colloidal silver is not removed from solution on exposure to the animals, there should have been an excess accumulation of this substance in the twice used water; but after again conditioning the used substance it was necessary to add the same amount, one drop of colloidal silver per 25 cc. to this suspension in order that it might be as deeply colored as was a fresh suspension made up with that amount of the colloidal silver. There was only sufficient colloidal silver left in this twice used suspension to discolor the liquid. Either the animals, or the aëration, or both, had removed the greater part of the silver, and it can be readily demonstrated that the effect was not wholly due to aëration. The water thus treated was distinctly sirupy, and evidently held organic matter, received from the two lots of *Ophioderma* which it had contained.

The effect of this twice reconditioned suspension was tested as before. Comparison tests were run with newly prepared suspensions, and the results are summarized in Table III.

TABLE III.

SHOWING RIGHTING TIME OF *Ophioderma* AFTER AN EXPOSURE OF 19 HOURS IN TWICE RECONDITIONED AND IN FRESH SUSPENSIONS.

Reconditioned Suspension.

Bunch.			Isolated.		
Number.	Mean Time.	Spread.	Number.	Mean Time.	Spread.
15	46″	10–221″	11 *	3° 27′	17″–22°
New Suspension.					
15	34″	10–175″	12	1° 30′	10″–14°

* One failed to give the righting reaction.

There is no evidence here of the presence of an auto-protective secretion in the sea water, protecting animals from the action of the colloidal silver, nor is there evidence for the presence of an active auto-destructive agent, which Drzewina and Bohn also postulated to explain certain cases in which the greater mass of animals exposed to KCl die more rapidly than do the solitary individuals. In the presence of an active auto-destructive secretion one would expect the mass to die more rapidly than do the isolated individuals, which is not the case.

There is good evidence that the secretion of slime or other organic matter into the suspension does remove the colloidal silver and so render the solution less toxic. It is particularly significant that after having been twice used, the suspension required as much colloidal silver as was used in a fresh suspension to bring it to the same color. Similar results were obtained from other experiments, and additional evidence on this point will be presented shortly in connection with another aspect of the subject.

The work of Bresslau is of interest here. He finds that protozoa give off a substance which he calls " tektin," a mucin-like body. This is given off in greater abundance when the animals are stimulated by heat, pressure, methylene blue or iodin, etc. The " tektin," when given off, takes up water rapidly and exhibits strong surface activity, adsorbing foreign particles readily. Bresslau tells of putting 2 cc. of liquid from a culture of infusoria (either *Colpidium* or *Paramecium*) containing many individuals in one dish and a similar amount from the same culture, but with few individuals, into another. Into each he introduced one cc. of one per cent. solution of methylene blue. Both produced the " tektin " and adsorbed this poisonous material, that with the many animals so much more completely that the possibility of surviving the toxic action of the poisonous material was greatly increased.

Concerning the Specificity of Mass Protection against Colloidal Silver.

Drzewina and Bohn (1921*a*) in discussing the protection furnished by masses of tadpoles of *Rana fusca* exposed to the action of colloidal silver, as compared with isolated individuals similarly treated, say: " Nous cherchons à préciser la nature des substances protectrices, at à voir en particulier si elles sont specifiques," but they do not present data on this point.

There are two types of specificity possible. The protective secretion may have no other function and so be specific in that sense or it may be limited in protection to the species producing it. The first type of specificity will be discussed later. The latter aspect was investigated by placing one animal in a restricted volume of water containing a number of animals of a different species. All our observations show that the protective action of the mass is not limited to a given species. This is what would be expected if the fixing of the colloidal silver in some manner is the principal element in the protective action. The results of typical experiments are summarized in Tables IV and V.

TABLE IV.

SHOWING THE NON-SPECIFICITY OF THE PROTECTION AGAINST COLLOIDAL SILVER.

Animals.	Water Cc.	Colloidal Silver Drops.	Time to Death, Hours.
Many *Cladocera*, one *Asellus*	12.5	8	Over 36
One *Asellus*	12.5	8	4.5
Many *Cladocera*, one *Planaria*	12.5	8	Over 36
One *Planaria*	12.5	8	5.5
50 *Asellus*, one *Planaria*	12.5	8	Over 36
One *Planaria*	12.5	8	5.5
Desiccated parotid gland, two *Planaria*	10	10	Over 36
Two *Planaria*	10	10	7.5
Snail slime, two *Planaria*	10	5	Over 36
Two *Planaria*	10	5	Less than 18
Snail slime, two *Planaria*	10	5	Over 36
Two *Planaria*	10	5	Less than 16
One *Physa*, two *Planaria*	10	5	Over 36
Two *Planaria*	10	5	11

TABLE V.

SHOWING COMMUNITY PROTECTION AGAINST THE ACTION OF COLLOIDAL SILVER.

Two drops of colloidal silver in 5 cc. pond water; exposure 13 hours, then washed and placed in pond water. Temperature 21°.

Animals.	Crawling.	Lived.	Died.
23 *Planaria maculata*	25	23	0
10 isolated *P. maculata*	0	1	9
25 *Dendrocœlum*, 1 *P. maculata*	1	1	0
30 *Glossophonia*, 1 *P. maculata*	0	1	0
26 *Asellus*, 1 *P. maculata*	1	1	0
25 *Segmentina*, 1 *P. maculata*	0	1	0
Pond moss, 1 *P. maculata*	1	1	0

As these tables show, such diverse organisms as *Cladocera, Asellus,* pond snails, pond leeches, *Dendrocœlum,* and even pond moss, if present in quantity, markedly protect planarians from the toxic action of colloidal silver. Even the actual presence of living organisms is unnecessary; snail slime without the snails protects efficiently apparently by adsorbing the colloidal silver. The slime becomes densely colored as the water becomes lighter in color. Suspensions in water of desiccated parotid glands of sheep exhibit similar adsorptive phenomena and have similar protective value.

A still more severe test of the specificity of the protection was made by placing recently killed *Asellus* in glass dishes containing 10 cc. of water plus two drops of colloidal silver, and introducing with these dead isopods one *Planaria dorotocephala*. At the end of 7 hours exposure, when 20 planarians isolated into a similar volume of the same concentration showed, 6, one third disintegrated; 13, one half disintegrated, and one wholly so, the five worms isolated into suspensions containing dead isopods showed one intact though bloated; 3, heads only, disintegrated; and one with both head and posterior end disintegrated. Thus the presence of the recently killed isopods decidedly protected the otherwise isolated worms from the toxic action of the colloidal silver. This, taken with all the other evidence at hand, furnishes convincing proof that the protective action of the mass against colloidal silver, extends beyond the species limits.

Discussion.

We have shown in the preceding pages that, within limits and other conditions being equal, there is greater protection the greater the mass of the animals present, when exposed to the same amount of colloidal silver in the same volume of water. Further, the protection is largely and perhaps completely, furnished by the fixation of the toxic substance by the mass of animals, so that each escapes receiving a lethal dose; while, with the same concentration, isolated individuals receive a stronger dose of the toxic substance. The colloidal silver may be differently fixed in different animals, but with those that secrete slime, like planarians, the colloidal silver is adsorbed on the slime. With other animals observed, it may be removed by adsorption on the surface of the animals themselves. Finally, as would be expected from this mechanism, we have demonstrated that the protection furnished by the mass, is, at least to a considerable extent, independent of the species present.

Our experiments do not support the favorite hypothesis of Drzewina and Bohn that group protection among these aquatic animals is furnished by the rapid production in the presence of a toxic agent, such as colloidal silver, of a more or less mysterious auto-protective secretion. It is true that the production of slime by planarians actually serves as an auto-protective agent, not only in fixing colloidal silver in these experiments, but very probably in protecting the planarians from sudden changes in culture or habitat water. But slime production cannot be regarded as a specific auto-protective secretion, either in the sense that it is used for no other purposes, for obviously it plays many other roles in the economy of slime producing organisms, or in the sense that slime is limited in its protecting power to the one species producing it, since the protection furnished by aggregations of mixed species is easily demonstrated.

It is obvious that our experiments do not demonstrate that even in these cases there is no production of a more subtle sort of auto-protective secretion. Conclusions on this point must remain tentative pending the completion of appropriate quantitative chemical determinations. These experiments do make it unnecessary to assume the production of such a substance in order to explain group protection in all cases. Even the experiments with dead isopods are not conclusive on this point. In these experiments, the presence of freshly killed *Asellus,* in numbers, furnished marked protection for the solitary planarians. The supposition can be advanced that this protection was due to the diffusion of some chemical from the dead bodies. These experiments do demonstrate that the presence even of other living organisms is unnecessary for protection from the toxic effect of colloidal silver. So far as that is concerned, any other inert substance capable of fixing the colloidal silver by adsorption or otherwise should have the same effect.

This general phenomenon of the uptake and fixing of dyes and other possibly toxic reagents is well known, particularly by plant physiologists, who have developed a considerable literature concerning the method of fixation of such substances by living plants (cf. Scarth). The same phenomenon has been described for animals, and the protective value it furnished to a mass of animals has been recognized particularly by Bresslau.

In their work upon the relation between mass of animals present and toxic reagents, Drzewina and Bohn found that in the presence of certain chemicals, principally KCl, members of the group died more rapidly than did isolated animals. They explain such results by postulating the production of an auto-destructive secretion. Their most impressive test for the presence of such an auto-destructive secretion was similar to that for the opposite effect, viz., the placing of a single individual into a solution in which a group of animals had died, and comparing the survival time of such an animal with one isolated into freshly prepared solution. We have no evidence to present on this point at present, but before accepting the hypothesis of the production of some specific auto-destructive secretion, we should need to examine the extent of the phenomenon, and the type of reagents that cause a marked increase in the destructiveness of the mass, particularly with regard to their effect on oxygen consumption and the elimination of waste products.

The deleterious effect of the crowding of many animals into close quarters has long been known and may be easily demonstrated. Goetsch has recently shown that different factors produce the unfavorable effect with different animals; thus, with *Hydra*, decreased food supply is most important; with *Planaria*, equally fed, the accumulation of waste products becomes the limiting factor; with tadpoles, the deleterious effect appears most closely correlated with frequent contacts between different members of the mass. Limitation of available oxygen has the same effect with all. Until it is demonstrated that chemicals such as KCl cannot produce the observed increase in the menace of the mass by their known effects upon one or all of these relations, it is idle to speculate concerning hypothetical auto-destructive secretions.

Our experience that protection is furnished by heterotypic aggregations as well as by those of the same species has important ecological implications. Hitherto biotic communities have been regarded as being organized by similar or dovetailing requirements which the organisms make of their environment. These may lead them to dwell in the same or in similar habitats, while animals with other requirements separate into a different community. Communities are also considered to be integrated by their food interrelations. (Forbes, Shelford.)

The work with colloidal silver, here reported, taken together with the observations of Bresslau and the experiences of the plant physiologists, warrants the postulation of another and more subtle integrating factor in animal communities; and, when the observed effects of pond mosses are remembered, in biotic communities also. This integrating factor might well be called the auto-protective value of the community. Further investigations concerning the extent of the phenomenon in nature are needed, but the indications are that the secretion of such substances as slime, and probably even the accumulation in the surrounding environment of a certain concentration of what are ordinarily called waste products, help render a raw environment more suitable for many forms.

Drzewina and Bohn found tadpoles resisting colloidal silver more readily if the solution were made up in pond water rather than in tap water. They interpreted this as being due to the presence of some dilute auto-protective tadpole secretion, but from our experience it seems more probable that the protection was due to the presence of slime or other material secreted by all pond animals.

It is a commonplace of ecology that many species of plants and animals so react upon their environment as to limit the continued existence of their species in that locality, while at the same time preparing the way for their successors. This general condition is well illustrated by the work of Woodruff on the sequence of protozoan cultures. We are proposing an extension of this principle to include the idea that the pioneers in a sucession first react upon their environment so as to render it more favorable for supporting themselves and their own associates. It seems probable that a continuation of the same processes and further accumulation of the same products that at first are favorable will ultimately become unfavorable and so help cause ecological succession.

Conditioning of a raw environment to support a population is not limited to accumulation of favorable chemicals, for recent work (Allee, 1927*a*) indicates that in the absence of customary physical elements, as of eel grass in the case of *Ophioderma,* the animals may so react with each other as to substitute their own bodies for the missing grass.

It must be admitted that to date, most of the data supporting this view depends on laboratory studies made largely upon reagents with which animals seldom or never come in contact in nature. Chemically clean glass dishes, filled with conductivity water given the proper osmotic value by the addition of chemically pure salts, and supplied with the optimal quantities of pure food do not furnish an optimum habitat for many animals, and it does not necessarily follow that the relations shown under such conditions would be duplicated in nature. There is, however, enough reason for thinking that similar relations may obtain in nature to justify further investigations.

Regardless of the protective mechanism, the fact that under certain conditions, masses of animals may have greater survival value for their components than would be the case if all were isolated, is of decided and general biological significance. In large bodies of water such aggregations might condition the water immediately around them, and in small bays where conditions become more pond-like, or in the more restricted area under small rocks, such conditioned water might have considerable permanence. In tide-pools, or in small streamside pools, the community auto-protection could easily have marked survival value. Even if it should appear that the only protection furnished is the distribution of the toxic agent among so many animals that none receive a lethal dose, the older conception that crowding is always a menace, hibernation and breeding season sometimes excepted, must be revised; and the indicated revision allows a better understanding of the persistence and probable significance of animal aggregations of a low level of integration.

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POLYVITELLINY IN POND SNAILS.

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INTRODUCTION.

The occurrence of more than one vitellus, or embryo, in a single egg of some of the fresh-water pulmonates has never been satisfactorily explained. Although various forms of abnormal development which might be considered to have some relation to twinning, such as a *Campeloma* having two separate dextral shells (MacCurdy, '09), have been reported, such abnormalities were not observed in our laboratory stock or among the many wild individuals examined. The fact that a wild snail, number 5, Table I., was found to lay eggs having more than one vitellus when brought into the laboratory, suggested the idea that the laying of such abnormal eggs might be a transmissible character. When, however, in less than two months several of these abnormal, or polyvitelline, eggs had been laid by the controls and by other wild and laboratory reared snails, the genetic explanation seemed less likely. Fortunately, some of the stock from the projected genetic experiment, the control and other isolated stock which was used in determining the viability of eggs laid by virgins (Crabb, '27*a*) furnish enough experimental data to show that the laying of polyvitelline eggs is not hereditary. Although the genetic experiment was discontinued, the occurrence of these abnormal eggs was noted in the records of other experiments.

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Material and Methods.

Eggs of *Physa sayii, Lymnæa stagnalis appressa, L. columella* and *L. palustris* were used in this investigation. Egg masses of *L. s. appressa* and *L. palustris* containing polyvitelline eggs were placed in 5 per cent. formaldehyde for several hours, or even months; then the abnormal eggs were removed from the mass and mounted in hollowed slides, in 2.5 per cent. formaldehyde, to facilitate studying and drawing the vitelli. After a few weeks in the stronger solution the eggs ceased to shrink appreciably and began to darken but are still transparent after having been in the fluid eighteen months. The albumen of the eggs fixed in the strong solution over night and mounted in the weaker solution has neither coagulated nor blackened, and the vitelli are as plump and bright as they were when the eggs were sealed up over two years ago (Fgs. 3, 12).

In every instance the eggs recorded for individuals of each species represent all that that snail, or group of snails, laid during the period of observation. Thus, so far as the observations go, the data on the occurrence of polyvitelline eggs is much more reliable than if the masses had been collected at random.

Normal and Polyvitelline Eggs.

Normal eggs of the snails herein described consist essentially of an outer membrane enveloping a mass of albuminous substance which surrounds a relatively small, usually excentric, vitellus or yolk. Thus the structure of a pond snail's egg superficially resembles that of a bird.

Morphologically polyvitelline eggs of *L. s. appressa* and *L. palustris* differ from normal eggs only as regards the number of vitelli, and the amount of albumen displaced by the supernumerary vitelli. There is as much variation in size between normal eggs (often of the same mass) as there is between normal and polyvitelline eggs. This is also true of the vitelli. Abnormal vitelli having the yolk protruding or flowing out, such as are shown in Figs. 3 and 11, and even cases of absence of vitelli, are occasionally found in eggs which to all other appearances are normal.

Several eggs have been found adhering together, but in most cases each one contained a single vitellus. In one instance (Fig. 7) two embryos are in a single egg while the other egg has none. In another case three eggs are joined together; the two end ones having no vitelli and the middle one having only two vitelli. The most remarkable instance of adhering eggs is one in which six normal eggs are joined in a string. However, there is nothing to indicate that polyvitelliny is dependent upon such adhesion of eggs.

The maturation and early cleavage stages are apparently normal in polyvitelline eggs having less than a dozen vitelli. In some eggs having a larger number many of the embryos pass the trochophore stage and some even reach the early shell stage before dying. The first maturation spindle is formed in a clear area or " well " in eggs which have been laid and moves to the periphery (Fig. 2) of the vitellus. The first polocyte migrates out into the albumen and the second remains attached, as has been shown is the case in normal eggs (Crabb, '27*a*).

We have never been able to hatch more than four snails from a single egg, but we have succeeded in repeating this experiment four times, and a fifth egg containing four embryos was killed and mounted. By tabulating the data available on the viability of polyvitelline eggs of *L. s. appressa* we find that of 53 eggs having two vitelli each, 39 hatched each vitellus, 8 hatched only one and 6 failed to hatch either vitellus. Of 5 eggs having three vitelli, 3 hatched all three, and 2 hatched only two of the vitelli. From 4 eggs containing four vitelli each, sixteen young were hatched. Of 116 *L. s. appressa* hatched from polyvitelline eggs not one was sinistral.

Occurrence of Polyvitelliny.

Twenty-one *Physa sayii*, fifteen of which were hatched from the same mass of eggs, deposited 348 masses containing 9,061 eggs, two of which had two vitelli each.

Of a large number of *Lymnæa columella* eggs taken at random only one contained more than one vitellus. This egg had two, one of which died in early cleavage and the other reached the gastrula stage.

Three F_1 and six F_2 of snail number 5, Table I., deposited a number of masses, which are not recorded in Table I., but none contained polyvitelline eggs.

Polyvitelline eggs occur most often in those masses which contain a large number of eggs (*i.e.*, 70 to 150 in *L. s. appressa*), and large masses are deposited by large, old individuals in the prime of life more often than small masses. This fact and the com-

TABLE I.

NORMAL AND POLYVITELLINE EGGS LAID BY TWENTY *Lymnæa stagnalis appressa*.

Individual snails designated 1$F_1$1–1$F_1$6 are F_1 progeny of snail No. 1. Likewise, those designated 4$F_1$1, 5$F_1$1 and 5$F_1$2 are the F_1 progeny of snails No. 4 and No. 5. Snails Nos. 1–4 and 11 were adults when collected from lakes near Ann Arbor, Michigan, while all the others were reared in the laboratory. All the F_1 individuals and those numbered 6–10 inclusive were reared in isolation. The masses for each individual are consecutive.

Individuals.	No. Masses.	No. Eggs.	Masses Containing Polyvitelline Eggs.	Polyvitelline Eggs.	Vitelli in Each Egg.
1	70	5,453	13	23	2(20 eggs), 3, 4, 4
1$F_1$1	13	844	6	14	2(8), 3(4), 5, 32
1$F_1$2	7	560	2	2	3, 3
1$F_1$3	6	364	1	1	3
1$F_1$4	6	491	1	3	2, 2, 3
1$F_1$5	6	443	0	0	0
1$F_1$6	5	441	1	1	2
2	50	2,506	5	7	2(6), 3
3	1	105	1	25	2(22), 3, 4, 4
4	1	64	1	2	2, 2
4$F_1$1	22	1,608	8	25	2(22), 3, 4, 4
5	7	466	5	19	2(16), 3, 3, *ca.* 26
5$F_1$1	20	1,631	14	131	2(39), 3(12), 4(11), 5(3), 7, 9(3), 45, 6–15(29), 15–30(32).
5$F_1$2	8	708	1	3	2, 2, 2
6	9	687	2	2	2, 2
7	8	497	0	0	0
8	4	246	0	0	0
9	20	935	0	0	0
10	7	487	0	0	0
11	14	1,327	6	15	2(15)
20	284	19,863	67	273	

TABLE II.

NORMAL AND POLYVITELLINE EGGS LAID BY ABOUT ELEVEN *Lymnæa palustris*.

The eggs in group 1 were laid by two snails kept in one aquarium; 2, eggs from an aquarium containing eight snails; 3, eggs laid by a single snail kept in isolation. The masses in each group are consecutive.

Group.	No. Masses.	No. Eggs.	Masses Containing Polyvitelline Eggs.	Polyvitelline Eggs.	Vitelli in Each Egg.
1.......	16	926	6	10	2(9), 3
2.......	91	4,824	1	1	2
3.......	21	1,189	2	4	2, 2, 3, 5
11......	128	6,939	9	15	

paratively low number of eggs examined is probably the reason why polyvitelline eggs were not recorded for snails number 1 F_1 5, 7, 8 and 10, Table I. In the case of *L. palustris* the three individuals designated as groups 1 and 3, Table II., were large adults, but in group 2 only one was a large adult at the time we began examining the eggs. The data relative to the size and age of the twenty-one *Physa sayii* are not sufficient to determine whether or not the size of the individual and number of eggs in the mass are correlated with the presence or absence of polyvitelline eggs.

DISCUSSION.

The facts set forth in this paper indicate that polyvitelline eggs may be expected to occur among the normal eggs of old *L. s. appressa* in the prime of life, but that the laying of these abnormal eggs probably is not a transmissible character. The question whether or not the two or more individuals hatched from the same egg are true twins, triplets, quadruplets, etc., has been raised. The paper of Hall ('25) appears to be the only available record of "twinning" in Mollusca. He found eggs of the tubiculous mollusc *Serpuloides vermicularia* containing two embryos which he considered twins. Newman ('23) holds that true twins arise from a single cell and that twinning is "essentially a phenomenon involving a physiological isolation of equivalent parts of the blastoderm and a regulation of the isolated or twinned regions into complete embryos." He also states that he has "never seen a reference to a case of twins or double monstrosity in Mollusca

. . ." and attributes this to the fact that cleavage is determinate in animals of this phylum. From his explanation and his review of the works of writers who claim that twins, at least in the forms they studied, are produced by a process of budding (Patterson), fission (Stockard) or fusion (Gemmill) it is evident that the process of twinning begins later than the first cleavage of the egg. However, it might be possible for two ova to fuse before either has undergone cleavage; whether this would produce twins or not is another question.

We have been unable to find anything to indicate that the supernumary individuals developed from pond snails eggs arose from a single vitellus. Although some four dozen ovotestes of *L. s. appressa* were sectioned (Crabb, '27*a*) not more than ten and seldom as many as four vitelli were found in the ovotestis and hermaphrodite duct in any one snail and in no instance were ova, and rarely sperms, found in the filliform part of the hermaphrodite duct (*i.e.,* craniad to the region "O," Crabb, '27*b,* Fig. 1) which in life bends around the gizzard. Thus it appears probable that while actively contracting the gizzard inhibits the passage of ova to such an extent that several ova accumulate in the enlarged part of the hermaphrodite duct and during a period of reduced activity of the gizzard all pass into the convoluted uterus at one time. In this, or some other way, a number of vitelli become enveloped by the albumen and the egg membrane which would normally cover a single vitellus. Often a mass of sperms is enclosed with the vitelli (Figs. 1, 5). Thus, probably by sheer accident, a polyvitelline egg is produced, and for this reason the young hatched from such an egg should not be considered true twins any more than should all the other young hatched from that mass of eggs.

SUMMARY.

That polyvitelline eggs do not contain true twins is shown by the following facts:

1. The vitelli are normally enveloped by the albumen and egg membrane before any of them have undergone the first maturation division (Figs. 2, 6).

2. The vitelli often occur in uneven numbers (Plate 1, Tables I., II.).

3. Motile embryos show no evidence of attachment to each other.

4. Each of the 116 young was a normal dextral individual, except for size, when hatched.

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Plate I.

Figs. 1–6 and 10–13 are of *Lymnæa stagnalis appressa* eggs; Figs. 7–9 are of *L. palustris* eggs. All figures except Fig. 2 were drawn with the aid of the camera lucida to the scale shown in the plate. The eggs represented by Figs. 3 and 12 were mounted in a very weak solution of formaldehyde in a slide having the pits so shallow that the covers flattened them somewhat.

Explanation of Figures of Polyvitelline Eggs.

1. An egg having four vitelli. The dotted line represents the extent of a mass of sperms in the albumen.

2. Five unreduced vitelli occurring in a single section through an egg. The first maturation spindle is shown in one ovum, and an aster of a first maturation spindle is shown in another.

3. An egg laid by a wild snail, showing seven vitelli. Yolk globules have been extruded from four of the vitelli.

4. An egg having seven vitelli in sixteen to thirty-two-cell stages.

5. An egg with three vitelli in early cleavage stages. The dotted line indicates the extent of a mass of sperms in the albumen.

6. An egg having seven vitelli which have not given off the first polar bodies.

7. *Lymnæa palustris* egg with two advanced embryos, one of which probably belongs in the small attached egg.

8. An egg having two vitelli, one of which is in the four-cell stage.

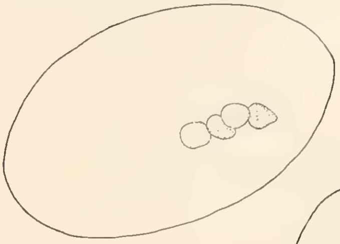
9. An egg having five vitelli in early cleavage stages.

10. *L. s. appressa* egg having five vitelli in early cleavage stages.

11. An egg having eight vitelli; one of which has disintegrated, while five are undergoing normal cleavage, and the other two apparently have not divided.

12. An egg having forty-six vitelli, laid by a wild snail.

13. An egg having twelve vitelli in four to eight-cell stages.



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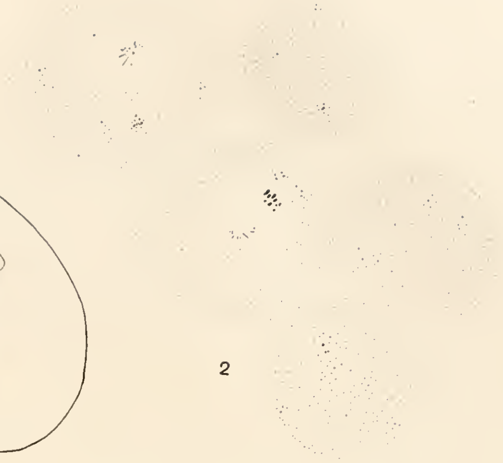

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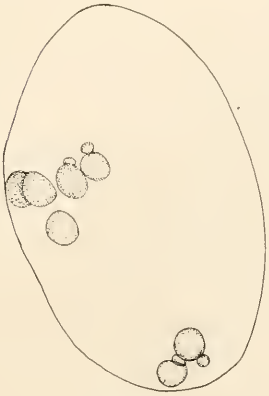

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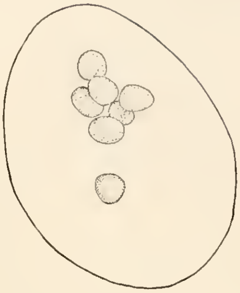

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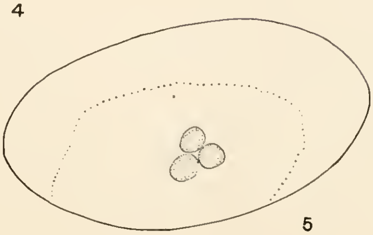

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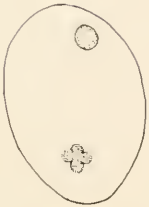

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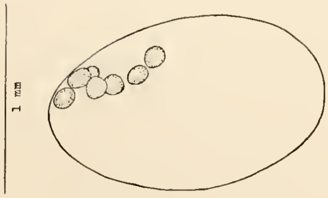

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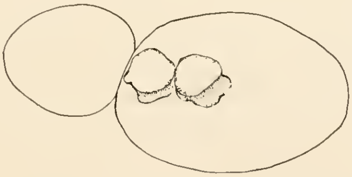

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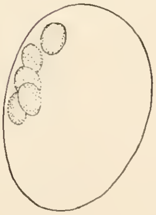

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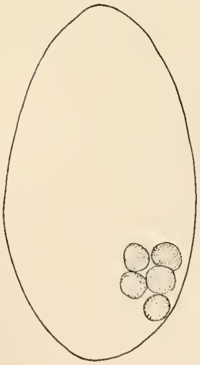

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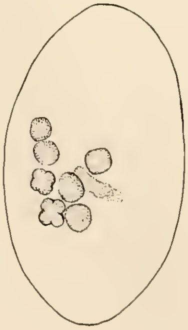

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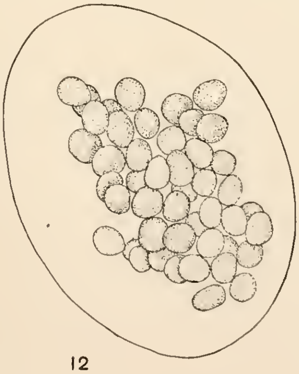

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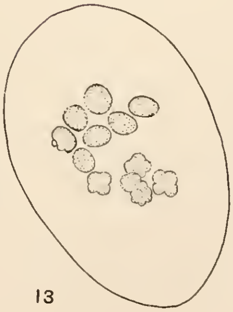

13

EDWARD D. AND RUBY M. CRABB.

THE EFFECT OF ALKALIES ON THE OXYGEN CONSUMPTION AND SUSCEPTIBILITY OF *PLANARIA DOROTOCEPHALA*.[1]

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I.

Introduction.

Considerable work has been done in this laboratory by Child and his students on the effect of chemical and physical agents on the modification of development and regeneration of organisms. For the interpretation of these results it is desirable that the action of the more commonly used chemicals on the rate of some metabolic process, such as respiration, be determined directly. Some work has already been accomplished along these lines, the effects of several substances on the rate of oxygen consumption having been tested: cyanides (Hyman, '19*a*), anesthetics (Buchanan, '22), caffein (Hinrichs, '24), and acids (Hyman, '25). The present paper is a contribution to this line of work and consists mainly of a study of the action of alkalies on the rate of oxygen consumption of *Planaria dorotocephala.* Hyman ('25) has already shown that acidification of natural water decreases the rate of oxygen consumption of *Planaria.* The question of the effect of increased alkalinity of natural water seemed of interest.

A number of investigations are available on the effect of increased alkalinity on various biological processes, such as activity, growth and development, and respiratory rate. Loeb ('98) found that the rate of embryonic development of *Arbacia* and *Fundulus* is accelerated in slightly alkaline solutions. Moore, Roaf, and Whitley ('05) also noted that bases in low concentrations favor growth and cell division in the fertilized eggs of *Echinus esculentus;* but Whitley ('05) failed to obtain any accelerating effect of alkaline solutions on the development of the teleost *Pleuronectes*. The fertilizability of *Arbacia* and *Asterias* eggs is increased by short exposures to alkaline sea-water according to Smith and Clowes ('24). Accelerations of activity by alkaline solutions have been observed by Dale ('13) in *Paramecium* and by Gray ('24) in the frontal cilia of *Mytilus edulis*.

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[1] Accepted as a thesis for the degree of Master of Science in the department of zoölogy of the University of Chicago.

Direct measurements of respiratory rate in alkaline solutions have yielded diverse results. In certain bacteria Brooks ('21, '22) noted the maximum rate of carbon dioxide production at or near neutrality with progressive decline in the rate with increasing alkalinity. The respiratory rate of the mold *Penicillium chrysogenum* is decreased 60 per cent. upon increasing the alkalinity of the medium from neutrality to pH 8.8 with NaOH (Gustafson, '20). According to Loeb and Wasteneys ('11) the rate of oxygen consumption of *Arbacia* eggs is increased 20–30 per cent. by raising the alkalinity of the sea-water with NaOH to pH 10.0 and increased 100 per cent. by raising it from pH 10.0 to 10.9. Similar results were obtained when ammonia was used. Thunberg ('09) found a decrease in the carbon dioxide production of excised frog muscle in solutions of NaOH, $Ca(OH)_2$, and $Mg(OH)_2$. Waldbott ('24) noted a slight acceleration of the respiratory metabolism of humans after the ingestion of alkaline solutions. The carbon dioxide production of tadpoles is increased in solutions of potassium and sodium hydroxide, but the rate of regeneration of the tail is retarded (Jewell, '20); Jewell regarded the increased respiratory metabolism as of a destructive nature.

From this review of the literature it is evident that both retardation and acceleration of biological processes result from exposure of organisms to alkaline solutions. In general, in the case of animal materials, an acceleration of growth and development and of respiratory metabolism in alkaline solutions has been found in the majority of cases.

Oxygen Consumption Experiments.

1. *Methods*.—The experiments consisted in determining the rate of oxygen consumption of planarians first in normal water

and then in water made alkaline to the desired degree by the addition of sodium or ammonium hydroxide. The general method of procedure was that described by Hyman ('19*a*). The species used was *Planaria dorotocephala*. Each experimental lot consisted of about 150 worms, 18–20 mm. long; these were selected from the laboratory stock and placed in a 500 cc. Erlenmeyer flask, in which they remained for a considerable time, being used in a number of experiments. They were fed in these flasks at intervals. At least three or four days were allowed to elapse after each feeding before the worms were used for experiment in order to avoid the increased respiratory rate consequent upon feeding (Hyman, '19*b*). The worms were fed sufficiently often to avoid any effects of starvation. From time to time new lots of worms were selected.

The water used was well water, an analysis of which is given by Hyman ('25). The alkaline water was prepared by adding enough sodium or ammonium hydroxide from stock solutions to raise this water to the desired alkalinity. The pH was determined with phenol red and thymol blue indicators by comparison with standard sets. At alkalinities greater than pH 8.6 a precipitate of calcium carbonate formed in the water. While there was no indication that this precipitate in any way affected the results, it was thought best to avoid it. This was done either by allowing the precipitate to settle and then decanting or by using carbonate-free water. This was prepared by adding 2 cc. concentrated HCl to eight liters of well-water and bubbling air through the water for 24 hours or more. That the rate of oxygen consumption of *Planaria dorotocephala* is the same in carbonate-free as in untreated well-water had previously been determined by Hyman ('25) and was verified in the present experiments.

Throughout each respiratory test, the flasks containing the worms and the blanks were kept in a large water bath at a temperature of 20° C. ± 0.5°. This was covered during the test. This darkening of the flasks together with the fact that the worms were kept continuously in the flasks was sufficient to eliminate movement. The worms remained very quiet throughout the oxygen consumption tests and the results cannot be ascribed to any differences in motor activity.

Two types of experiments were carried out: short exposures of a few hours to alkaline solutions, and long exposures of a week or more. Three experiments, that is, three flasks of worms, were generally carried on simultaneously.

2. *Short Time Experiments.*—In these experiments the rate of oxygen consumption of the worms was tested for two successive hours in normal well-water, pH 7.6 to 7.8. The water was then made alkaline by adding NaOH in varying amounts, giving a pH of 8.0 to 9.2 by intervals of 0.2 pH, or by adding NH_4OH similarly to alkalinities varying from pH 8.0 to 8.8. The rate of oxygen consumption was then tested in this alkaline water during the first third, and generally also fifth hours of exposure to it, freshly made alkaline water being used for each determination. As stated above, carbonate-free water was employed for alkalinities greater than pH 8.6. Twenty-three experiments were performed with sodium hydroxide and twelve with ammonium hydroxide. Table I. gives a typical experiment with each pH used for each of the two alkalies. The average per cent. of increase is based on all of the figures obtained in the alkaline solution. Table II. presents a summary of all of the short time experiments showing also the minimum, maximum, and average change in respiratory rate These tables show that the respiratory rate was sometimes decreased in alkaline solution but was more generally accelerated The normal variation in respiratory rate, based on twenty experiments where the respiration was determined in normal water for two successive hours, was 7 per cent. Only figures showing more than 7 per cent. alteration of the respiratory rate are therefore significant. From Table II. it can be seen that the higher alkalinities give generally a significant acceleration of the rate of oxygen consumption.

The worms would not survive alkalinities greater than those given in the table. They would live indefinitely in water made alkaline by NaOH to pH 9.0, but only twenty-four hours at pH 9.2. They were immediately killed in NH_4OH at pH 9.0. These results indicate that the action of alkali cannot be attributed solely to the hydroxyl ion.

It should be stated that the oxygen consumption of *Planaria* is independent of the oxygen content of the water at all ordinary oxygen concentrations (between 7.0 and 2.0 cc. per liter, at least). The oxygen concentrations employed in the experiments was such (5 to 7 cc. per liter) that there is no possibility that the reduction in oxygen content by the worms during the experiment could have the slightest effect upon the amount of oxygen consumed.

TABLE I.

RESULTS OF A TYPICAL EXPERIMENT, SHORT TIME, AT EACH pH USED.

Figures represent cc. of oxygen consumed per hour.

	NaOH						NH$_4$OH			
Normal respiration in water. pH 7.6–7.8.										
	0.21	0.24	0.21	0.22	0.26	0.26	0.29	0.26	0.26	0.31
Respiration in alkaline water.										
pH	8.0	8.4	8.6	8.8	9.0	9.2	8.0	8.4	8.6	8.8
1st hr.	0.23	0.26	0.33	0.26	0.27	0.33	0.29	0.28	0.29	0.39
3d hr.	0.25	0.30	0.24	0.28	0.33	0.34	0.30	0.26	0.26	0.37
5th hr.	—	—	—	—	0.30	0.30	—	0.30	0.28	0.39
Average per cent. of increase of each of above.										
	14	16	32	22	15	20	1.5	7.6	6	23

TABLE II.

AVERAGE PERCENTAGES OF INCREASE AND DECREASE BASED ON TOTAL OF ALL SHORT EXPERIMENTS DONE AT EACH pH.

Figures are ± per cent. from the normal.

	NaOH						NH$_4$OH			
pH	8.0	8.4	8.6	8.8	9.0	9.2	8.0	8.4	8.6	8.8
Total no. exp.	3	5	3	6	3	3	3	3	3	3
Increase and decrease in respiration rate from normal.										
Min.	−12	−12	+11	− 4	+ 3	− 7	−20	−18	−10	+19
Max.	+19	+28	+61	+86	+26	+58	+ 3	+15	+17	+32
Av. of all exp.	+ 6	+14	+32	+22	+17	+33	− 7	+ 3	+ 5	+25

The results of all of the short time experiments are given in graphic form in Fig. 1, made from the data given in Table II. The rate of oxygen consumption is given on the ordinate, normal respiration in untreated water being taken as 100, respiration in alkaline water as per cent. decrease or increase from this. The alkalinity in terms of pH is given on the abscissa. The solid line represents the results with NaOH, the dashed line with NH_4OH. As already explained only differences greater or less than 7 per cent can be taken as significant. In the case of NaOH the experiments at pH 8.4 to 9.2 show on the average an increase above the normal variation, but there is apparently no relation between amount of acceleration and degree of alkalinity. The drop at pH 8.8 and 9.0 is probably not significant. In the case of ammonia the curve rises steadily with increasing alkalinity but probably only the increase at 8.8 is significant.

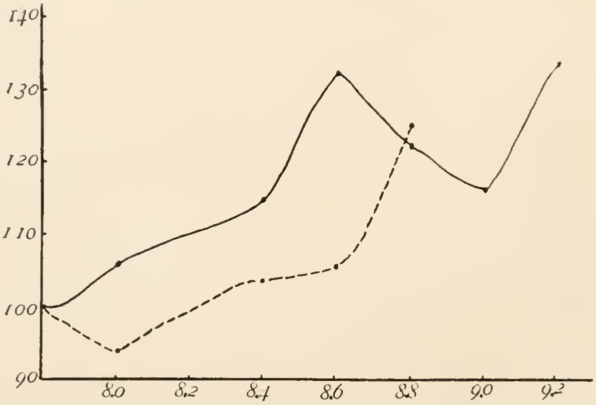

FIG. 1. Graph compiled from the average percentages of increase and decrease in respiration rate of all the short time experiments. See Table II. Per cent. of increase or decrease, normal respiration being taken as 100, on the ordinate and pH on the abscissa. Heavy line shows experiments in NaOH and broken line the experiments in NH_4OH.

3. *Long Time Experiments.*—These experiments consisted in determinations of the rate of oxygen consumption at frequent intervals in worms exposed continuously to alkaline water for periods of one to two weeks. Considerable difficulty was encountered in these experiments because the water would not remain at a definite alkalinity on account of the carbon dioxide given off by the animals. Various methods of keeping the water alkaline were suggested and were tried out. The carbonate-free water could not be used in the long time experiments, as, being unbuffered, it quickly became acid from the carbon dioxide given off by the

animals. Buffering this water with borax proved effective as a means of maintaining it at a definite alkalinity but the borax killed the worms in several days. Bubbling air through the flasks in the hope of removing the carbon dioxide emanating from the animals was not effective. When all of these methods failed, it became necessary to use the ordinary well water and to change it at frequent intervals. This proved fairly satisfactory. Boiling this water to remove dissolved carbon dioxide was of some help. The oxygen consumption of the worms was determined first in untreated well-water, pH 7.6 to 7.8. The water was then made alkaline to pH 9.0 with NaOH, decanted from the precipitate of $CaCO_3$ when necessary, and the worms kept in such water for one to two weeks continuously. The water was changed two or three times in 24 hours. Between changes, it became somewhat less alkaline but never fell below pH 8.6 throughout these long time experiments. The rate of oxygen consumption was determined every other day or every third day, always of course at pH 9.0. Owing to the fact that the worms lost weight during the experiment, because of starvation, for they were not fed throughout the period of experiment, it was necessary to weigh them in order to compare the rate of oxygen consumption at different intervals. The figures therefore represent the cc. of oxygen consumed per gram per hour. At the end of the experiment, the respiratory rate in normal water was again tested.

The results of three long time experiments which lasted one week are given in Table III., and of three more which lasted two weeks, in Table IV. In Table III., the oxygen consumption was determined first in normal water, then immediately in alkaline water (ph 9.0), then every other day in the alkaline water, and finally on the seventh day after the last test in alkaline water, again immediately in normal water. As shown in the table there was a marked acceleration of the respiratory rate during the latter part of the stay in alkaline water; this acceleration was immediately lost on return to untreated water, on the seventh day. The data in Table IV. give a similar result: acceleration during the exposure to alkaline water, immediate drop on return to untreated water.

TABLE III.

TABLE SHOWING RESULTS OF LONG TIME EXPERIMENTS IN BOILED WELL WATER, pH RAISED TO 9.0 WITH NaOH.

The pH never went below 8.6. Results expressed in cc. oxygen consumed per gram of animals per hour.

	1.	2.	3.	Av. % Increase 3 Exp.
I. day. Water pH 8.0	0.32	0.30	0.29	Normal. 100%
I. day. NaOH pH 9.0	0.28	0.28	0.30	− 5.5
III. day. NaOH pH 9.0	0.28	0.29	0.29	− 5.2
V. day. NaOH pH 9.0	0.44	0.26	0.41	+ 22.0
VII. day. NaOH pH 9.0	0.39	0.37	0.45	+ 33.0
VII. day. Water pH 8.0	0.24	0.26	0.25	− 17.0

TABLE IV.

TABLE SHOWING RESULTS OF LONG TIME EXPERIMENT IN ORDINARY WELL WATER DECANTED OFF PRECIPITATE.

The pH was raised to 9.0 with NaOH. The pH never went below 8.6. Results expressed in cc. oxygen consumed per gram of animals per hour.

	1.	2.	3.	Av. % Increase 3 Exp.
I. day. Water pH 8.0	0.21	0.18	0.19	Normal. 100%
I. day. NaOH pH 9.0	0.23	0.20	0.21	+ 10
III. day. NaOH pH 9.0	0.29	0.26	0.28	+ 43
VII. day. NaOH pH 9.0	0.24	0.23	0.24	+ 22
X. day. NaOH pH 9.0	0.24	0.23	0.21	+ 17
XIV. day. NaOH pH 9.0	0.27	0.29	0.23	+ 36
XIV. day. Water pH 8.0	0.23	0.20	0.18	+ 5

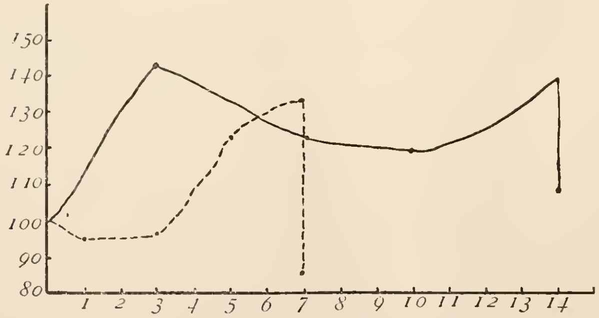

FIG. 2. Graph compiled from the data presented in Tables III. and IV. showing the results of long exposures of worms to NaOH solution, pH 9.0–8.6. Per cent. of increase or decrease, normal respiration being taken as 100, on the ordinate and number of days on the abscissa. Heavy line represents experiment in ordinary water and broken line experiment in boiled well water. The drop on the last day in each experiment shows the drop in respiration from NaOH solution to water.

The data given in Tables III. and IV. are graphed in Fig. 2, the solid line representing the data of Table IV., the dashed line, of Table III. The graph shows an acceleration of the rate of oxygen consumption during the greater part of the exposure to alkali, with an immediate drop at the end of each experiment on return to normal water.

Susceptibility Experiments.

The susceptibility method of determining differences in oxidative rate in different parts of the same organism and between different individuals of the same species was devised by Child and has been used extensively in this laboratory by him and his students. The method is explained by Child ('24). Briefly, the organisms to be compared are placed in toxic solutions of proper concentration or are exposed to lethal conditions and the various stages in disintegration are recorded. In general, the higher the oxidative rate, the shorter is the time before disintegration begins and the more rapidly does the disintegration progress. The method is therefore a rough means of comparing the relative rates of respiratory metabolism of comparable animals. Since the experiments on oxygen consumption had shown that the respiratory rate of *Planaria* is increased in alkaline solutions it seemed of interest to determine whether or not worms so accelerated would be more susceptible to chemical and physical agents than those respiring normally in ordinary water. A few experiments were tried to test out this point. Worms which had been kept for several hours in water made alkaline to pH 9.0 with NaOH were compared as to their susceptibility to toxic solutions and conditions while still in alkaline solution with control worms kept and tested in ordinary water or in solutions made up in ordinary water (pH about 8.0).

Comparison of susceptibility differences between different lots of worms is best accomplished by recording the progress of disintegration in certain arbitrarily selected stages. In the following experiments seven stages of disintegration were chosen: (1) worm entirely intact; (2) disintegration at the margins of the head; (3) from the end of marginal disintegration to complete disintegration of the head; (4) from the end of stage 3 to completion of disintegration half way back to the mouth; (5) from the end of stage 4 to completion of disintegration to the level of the mouth; (6) from the end of stage 5 along the margins to the posterior end of the first zooid; (7) from the end of stage 6 to complete disintegration of the first zooid. The death of the secondary zooids is not considered. Ten animals are usually employed in each test, and the number of animals in each stage of disintegration is recorded hourly. The results are graphed by giving to each stage a numerical value, beginning with zero for stage 7, complete disintegration, and adding ten for each stage, intact animals being assigned the value of 60. The numerical value assigned to each stage is then multiplied by the number of animals in each stage at each observation. These values for the ten animals are then added together and divided by ten; the quotient thus gives the average stage of disintegration of the ten animals at the particular time of observation. Thus if at one observation, three animals were in stage 2, four in stage 3, and three in stage 4, their values would be 100,160, and 120, respectively, making a sum of 380; this divided by ten gives 38, or the average stage of disintegration reached, at the time in question.

1. *Experiments with Chemicals.*—To test the effect of alkalies on susceptibility to toxic chemical solutions, it was necessary of course to choose chemicals that were in themselves neutral. Anæsthetics were selected as the most convenient. Ethyl alcohol, 4 per cent., and chloretone, 0.1 per cent., were used. The anæsthetics for the control set of worms were made up in ordinary well water, those for the experimental set in the same water, made alkaline to pH 9.0 with NaOH. The results are shown in Figs. 3 and 4, being graphed according to the method explained above. Fig. 3 represents the results with alcohol, Fig. 4 with chloretone. In each graph, the solid line is the rate of disintegration of the control worms in ordinary water, the dashed line, the experimental worms in alkaline water. In each case, the disintegrating action of the anæsthetic is seen to be more rapid in alkaline solution.

2. *Experiments with Lack of Oxygen.*—When chemical agents are used the question of their penetrability is involved and complicates the interpretation of the results. For this reason it was thought advisable to kill the animals by other than chemical means

and determine the effect of alkalinity on the time of death. Lack of oxygen was one condition that seemed suitable for a test. A petri dish was divided into three compartments by two paraffin walls. A powder made by grinding up pyrogallic acid and sodium hydroxide was placed in the center between the two paraffin walls. On one side of this were placed ten to twenty worms in ordinary water (pH 8.0); and on the opposite side in the third compartment, an equal number of worms of the same size and physiological

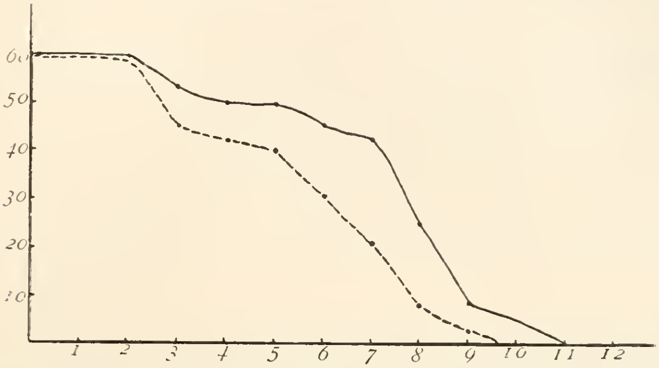

Fig. 3. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline alcohol (broken line) and worms not exposed to alkali and tested in neutral alcohol (heavy line). Alcohol used was 4 per cent. Hours on the abscissa and stages in disintegration on the ordinate. (See text for stages.)

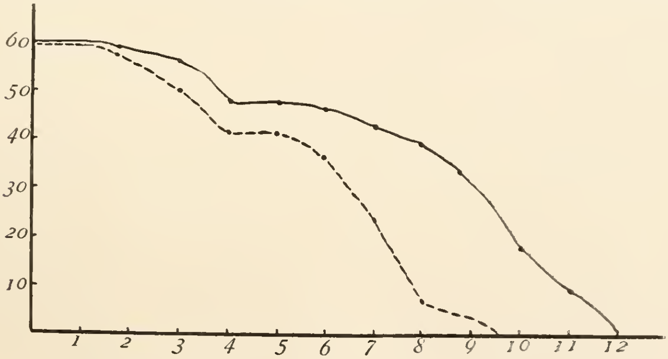

Fig. 4. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline chloretone (broken line) and worms not exposed to alkali and tested in neutral chloretone (heavy line). Chloretone used was 0.1 per cent. Hours on the abscissa and stages in disintegration on the ordinate. (See text for stages.)

condition in alkaline water (pH 9.0). A cover was sealed on airtight. As the oxygen was absorbed by the alkaline pyrogallate, the worms began to die. The results of one such experiment are graphed in Fig. 5, the solid line representing the control worms, the dashed line, the experimental worms. In several experiments of this kind it was always found that the worms died faster from lack of oxygen at pH 9.0 than at pH 8.0.

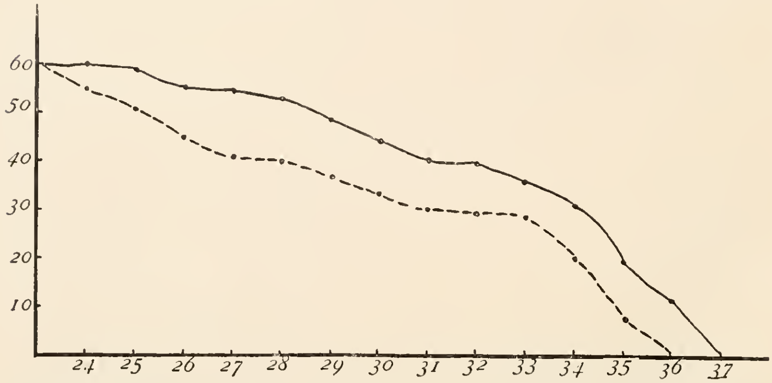

FIG. 5. Graph of disintegration gradient of worms exposed to NaOH solution and tested in alkaline solution in lack of oxygen experiment. Heavy line represents worms in ordinary water and broken line worms exposed to alkali. Record was not taken until disintegration began, *i.e.*, the twenty fourth hour. Hours are on the abscissa and stages of disintegration on the ordinate.

3. *Experiments with Ultraviolet Radiation.*—Comparable lots of worms were exposed to ultraviolet radiation from a Cooper-Hewitt machine for a duration known to be lethal, one lot at pH 8.0, the other at pH 9.0. The worms were continually agitated during the exposure to insure uniform radiation. In all trials, the worms in the more alkaline water died more rapidly than those at the lower alkalinity. Death of course occurs only some hours after exposure to the radiation.

These experiments indicate that planarians are more susceptible to toxic chemicals and to lethal conditions when exposed to them in water of increased alkalinity, which is not in itself injurious.

4. *Susceptibility to Alkali.*—The question was raised whether the worms are able to acclimate to increased alkalinity. The long time experiments did not suggest that this was the case within two weeks' exposure, since the acceleration of the respiratory rate

endured through the period of the experiments. A further test by the susceptibility method was suggested. If any acclimation occurred, worms that had been kept in a non-injurious concentration of alkali should be less susceptible to a lethal concentration of alkali than control worms. Stocks of worms were kept in water made alkaline to pH 8.8 and 9.0, respectively, with NaOH, and were compared as to susceptibility to a higher concentration of NaOH with control worms living in ordinary water, pH 8.0. Tests were made every third day over a period of ten days. No difference in susceptibility between experimental and control worms was found. The resistance of *Planaria* to alkali was, therefore, not increased by continuous exposure, up to ten days, to alkaline water.

Discussion.

The experiments reported in this paper show that in general the rate of oxygen consumption is accelerated in water made more alkaline than normal, within physiological limits, by sodium or ammonium hydroxide. This increase in the respiratory rate lasts as long as the worms remain in the alkaline water, at least up to two weeks. Upon return to water of normal alkalinity, the respiratory rate drops at once. No evidence of any acclimation to the alkaline environment appeared in the course of the experiments. The oxygen consumption remained at a supernormal figure during continued exposure to the increased alkalinity. Susceptibility tests also showed no increased tolerance to alkali as a result of living for some time in water of increased alkalinity.

The question of the cause of the accelerated respiratory rate in alkaline water is of interest but the present experiments throw no light on the matter. It is rather generally accepted that alkalies increase permeability or have some other surface action (*e.g.*, Osterhaut, '14, and Warburg, '10). Such surface changes might well be the cause of the acceleration of the respiratory rate. It is not probable that penetration of the alkali into the interior is a factor in the acceleration for although ammonia penetrates cells readily it is believed that sodium hydroxide does not penetrate until the surface is actually injured. Since both alkalies caused an increase in the rate of oxygen consumption, the effect appears to be a surface one.

Not only is the oxygen consumption of *Planaria* increased with exposure to alkaline solutions but also the susceptibility to toxic agents and conditions is greater when such agents and conditions are applied in alkaline water. In the case of chemical agents, this increased susceptibility in alkaline solution might be ascribed to increased permeability. This explanation does not seem applicable, however, to the result with lack of oxygen and ultraviolet radiation. Exposure to these conditions is more rapidly lethal in water of increased alkalinity than in normal water. It seems necessary to conclude that the increased susceptibility of worms to chemicals and to toxic conditions when exposed in water of increased alkalinity is in some way associated with the accelerated metabolism of the animals in alkaline water. Susceptibility is thus again indicated as a method of measuring roughly differences in general metabolic rate.

Summary.

1. The general result of exposing *Planaria dorotocephala* to water whose alkalinity is increased from pH 7.6 or 7.8 to 8.0 to 9.2 by addition of NaOH or to 8.0 to 8.8 by addition of NH_4OH is an increase in the rate of oxygen consumption, whether the exposure is for long or short periods.

2. The increase lasts as long as the planarians remain in the alkaline water (longest experiment, two weeks). A return to the normal or to a lower rate (probably result of starvation) occurs at once when the animals are returned to water of the original pH.

3. The resistance of planarians to lethal concentrations of alkali is not altered by long exposure (ten days) to non-injurious concentrations of alkali.

4. The susceptibility of planarians to toxic chemical solutions, to lack of oxygen, and to ultraviolet radiation is greater when they are exposed to these conditions at pH 9.0 than when exposed at ordinary alkalinity of normal water (pH 7.8 to 8.0).

I desire to express my thanks to Professor C. M. Child and Dr. L. H. Hyman under whose direction the work was done.

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A CASE OF APPARENTLY ADAPTIVE ACCELERATION OF EMBRYONIC GROWTH-RATE IN BIRDS.

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Temperature experiments upon the egg of the domestic fowl have shown that the period of incubation varies inversely with the temperature at which the eggs are incubated. It may be assumed that this is due to an increase in the increment of embryonic development. If we consider any temperature well within the margins of safety for the egg (high 105° F. *ca.*, low 100° F. *ca.*) such as 102.5° F., we may say that an increase of one degree leads to an accelerated development while a decrease of one degree leads to a depressed or inhibited development. However, as far as I have been able to discover, no case is known in nature where an adaptive acceleration of the rate of embryonic development seems to have accompanied the evolution of new species within a group. The purpose of this paper is to report on what seems to be such an instance.

The cowbirds are a group of icterine birds comprising half a dozen species placed in three closely related genera:—*Agelaioides, Molothrus,* and *Tangavius.* Of these three, the first named is in every way the most primitive and doubtlessly represents the primitive stock from which the other two have evolved. The last mentioned is a side branch of the cowbird group and need not be considered in this paper. The species of *Molothrus* (and *Tangavius*) are parasitic in their breeding habits, *i.e.,* they do not make any nests of their own but deposit their eggs in nests of other birds and leave them to be incubated and hatched by these strange species. *Agelaioides* is not parasitic but is not entirely normal in its breeding habits. It breeds in old nests of other birds but cares for its own eggs and young. It will build for itself only if it cannot possibly get possession of a nest already built, showing that it still possesses some of its original nest-building instincts, but makes use of them only in case of emergency. In this genus the female is extremely shy when on the nest and were it not for the protection of the male it is doubtful if in many cases the female would take sufficient care of the eggs. In *Agelaioides* the incubation period is 12½ to 13 days.

The genus *Molothrus* contains three species all of which are parasitic but have the parasitic habit developed to unequal degrees of perfection. The stages of perfection of the parasitic habit in these birds agree with what seems to be the phylogenetic relationships of the species. The most primitive member of the genus, *M. rufo-axillaris,* is parasitic on the still more primitive, non-parasitic *Agelaioides badius.* Its incubation period is the same as that of its victim and varies from 12½ to 13 days. The second species, *M. bonariensis,* is parasitic on a large number of small birds but has the parasitic habit very imperfectly developed, wasting large numbers of its eggs by laying in deserted nests, or even on the ground. The incubation period of this species is 11½ days. The third species of the genus, *M. ater*, has the parasitic habit best developed, lays its eggs in the nests of a great many species of birds, and does not waste its eggs as does *M. bonariensis.* This species has an incubation period of from 10 to 10½ days. (No bird in the world is known to have a shorter incubation period; few have one as short.) [1]

For the successful prosecution of a mode of reproduction such as parasitism implies it is obviously of great importance to the parasitic species to have a short incubation period as its egg may be laid in a nest after incubation has been started in the other eggs and yet must hatch as soon if not sooner than these others if the young parasite is to survive. (As a matter of fact in the majority of cases, irrespective of the species of bird victimized, the cowbirds' eggs hatch first.) Consequently when we find that with the perfection of the parasitic habit in the cowbirds (from the primitive non-parasitic *Agelaioides badius* to the relatively perfect *Molothrus ater*) there is a corresponding diminution of the period of incubation, or in other words, an acceleration of the embryonic growth-rate (amounting to about 20 per cent. difference between *A. badius* and *M. ater*) it is difficult to interpret the case as other than adaptive. Shortness of incubation period could very easily have been of enough selective value for natural selection to operate on in a very decided fashion.

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[1] The evidence on which the phylogenetic relationships of the species of cowbirds is based is contained in a manuscript now going the rounds of the publishers.

Lest it be thought that the differences in incubation period of the various species of cowbirds are due to differences in the extent of embryonic development, it should be noted that all of these birds hatch out in exactly the same stage of development. The size of the bird incubating the eggs seems to have no noticeable effect on the period of incubation. Birds as large as mockingbirds and as small as vireos hatch out young cowbirds in the same number of days. The size of the eggs of the various species of cowbirds is practically the same in all cases.

COMMENSAL ASSOCIATION OF A SPIDER CRAB AND A MEDUSA.

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During four successive years the writer was associated with a week-end biotal survey of the coast of South Carolina, and on two of these field studies encountered a curious commensal relationship between a spider crab and a medusa. The animals concerned were *Libinia dubia* Milne-Edwards and *Stomolophus meleagris* L. Agassiz, both of them common and typical inhabitants of the littoral zone of the Austroriparian Subregion.

Libinia dubia is a spider crab of moderate size with a rounded carapace averaging 6 cm. in diameter. It has long and slender walking legs and six median dorsal spines. Adult stages are confined entirely to the bottom zone where they crawl about in search of food. They are scavengers and members of the benthos.

Stomolophus meleagris is a rhizostome medusa of a diameter of 18 to 20 cm., hemispherical, without marginal tentacles, with eight rhopalia, and with fused oral lobes which form a thick cylinder, at the bottom of which are eight pairs of frilled lobes and a small central mouth opening. The margin of the umbrella is colored with a dark reddish-brown material, fading out aborally, which comes off freely and stains the hands when the specimens are picked up for inspection. They do not appear to possess any nettling organs, or at least none which could be employed defensively. This form is by far the most abundant Scyphozoan of the South Carolina coast, and is one of the more conspicuous planktonic organisms of the littoral zone.

Here we have to do then with a commensal relationship between a member of the benthos on the one hand and of vertically the most distant life zone, the plankton, on the other—certainly a most unusual arrangement. By means of this alliance the crab becomes a transient component of the plankton. The association

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consists in the presence of the crustacean within the subumbrellar space of the medusa, clinging to the manubrium. Most of the crabs were observed with the head directed downward, but a few were situated with the head upward. In no case was more than one crab found in a single medusa. They resisted detachment, holding on tightly by means of the sharp and strongly curved ends of their legs, and would occasionally attempt to escape by scrambling around the manubrium, just as a squirrel does around a tree trunk. In such cases, however, their agility was not marked.

At Georgetown and at Murrell's Inlet, S. C., in the north-central region of the coastline, no examples of commensalism were observed. One *Libinia dubia* was taken on the rocks of the jetties at Georgetown, but no living medusæ were seen at either station. At Bluffton and at Sullivan's Island, in the south-central portion, the following records were obtained: Bluffton, three medusæ seined from relatively deep water, each containing a crab; no other examples of either species were seen. Between Sullivan's Island and the Isle of Palms, near Charleston, seventeen *Stomolophus* were taken by net and by hand from shallow water, and of these sixteen concealed a *Libinia*. In the remaining case, a crab was found on the bottom in the immediate vicinity of the jellyfish and the collector believed the crab to have just dropped from his conveyor, probably due to the commotion in the water which the numerous members of the party were causing. No other examples of either species were encountered, so this supposition is strongly probable. The commensal crabs varied in size but were all fully adult. At all four stations great numbers of stranded medusæ, mostly *Stomolophus* were seen along all beaches.

Thus it is seen that (1) along the South Carolina coastline both *Stomolophus* and *Libinia* are not uncommon and are frequently abundant; more so in the southern half, and especially true of the medusa as attested by the many cast-up specimens. (2) With a single exception, all examples of *Libinia dubia* captured were taken from the subumbrella of the medusa. (3) Again with a single exception, and that one a doubtful case, all examples of *Stomolophus meleagris* were found to harbor a crab. (4) All of the observations were made in the month of May and involved living adult stages of the commensals.

Several varieties of Hyperid Amphipods and also certain small fish are commonly known to seek shelter beneath the umbrella of various medusæ, but cases of large sized crabs occupying this situation seem to be rare. No references in the literature involving either of these types of animal as commensals were found by the writer, who is indebted to Dr. Mary J. Rathbun for the two following citations: Rathbun, '04, in writing of the distribution of Cancer jordani in Monterey Bay, California, notes " one under rocks between low tide mark and mean tide, and three from the subumbrella space of a large violet-and-white jellyfish " (species not stated). Weymouth, '10, writes: "*Cancer gracilis* is represented by a considerable number of specimens, both young and adult, all obtained by dredging, though in Puget Sound it is an abundant shore crab. On several occasions the young of this species has been found in considerable numbers clinging to the subumbrella of various medusæ. These have all been of small size—5 to 10 mm.—but it was not until the summer of 1908 that younger stages were found. On one occasion a number of medusæ were collected and in examining these many very young crabs were obtained and a smaller number of megalops. Some of these were kept alive until the molt to the young crab stage took place, so that there can be little doubt that the specimens were really the megalops of *gracilis*. Later megalops were obtained from other species of medusæ, and crabs of a slightly larger size than those found on the jellyfish were dredged in considerable numbers; it would appear, therefore, that in the case of *gracilis*, at least a considerable number of individuals pass that portion of their life history from the end of the free-swimming stage, probably early megalops, until reaching a size of 15 to 20 mm. clinging to medusæ, after which they drop to the bottom and live in the manner of the adult. I have found no other species than *gracilis* on medusæ though Miss Rathbun reports one specimen of *jordani* from the same situation. It would be interesting to know if this form of life history were universal with *gracilis* and if it were common in any other species." It will be noted that Weymouth is incorrect in the number of specimens observed as commensal by Dr. Rathbun, and also that no identifications of the medusæ involved are given.

The conclusion reached by Weymouth as to the cause underlying the association he reports is entirely plausible, but what is one to say in the case of *Stomolophus* and *Libinia?* It does not seem possible that either commensal was concerned with the food habits of the other; no crabs were observed feeding on dead specimens of the medusa, and though they may do so in deeper waters, this type of crustacean is not known to attack living prey. It is certain that the crab added the burden of freight to the jellyfish, but probably did it no other injury: also any possible benefit to the latter seems unlikely. On the other hand it is unquestionable that the crab received both shelter and transportation, though whether either or both of these benefits accounts for the condition is problematical. That the association has anything to do with the life history of the crab appears doubtful, since all specimens found were adults and long past the delicate stages of *Cancer gracilis* collected by Weymouth. The only other speculation which occurs to the writer is that the crab might resort to such shelter at the molting periods, but here again fact does not support theory, as those individuals observed all had fully hardened shells. The purpose of this curious union then remains to be determined by more extended and exact studies.

Another interesting angle of this situation is the problem of how the crab attains the medusa. Since the former is absolutely confined to the bottom in so far as its own efforts are concerned, there remain but two alternatives: either the medusa must descend to the substratum at least occasionally, and for an obscure purpose, or else one of the larval stages of the crab must seek shelter within the umbrella and then remain attached during a long period of its mature life, for a reason equally difficult to conjecture.

And if the facts of the case are not sufficiently strange, consider also the circumstance that this observation has so long escaped scientific detection and is also unknown to all of the local fishermen, boatmen, and sportsmen whom the writer interviewed, and this in spite of the familiarity of the animals concerned and the well nigh universality of the association in the region covered.

Spider crabs are famous for their protective adaptations, adorning the carapace with algæ, sponges, and hydroids, or possessing a shell so colored and sculptured as to blend effectively with the environment. Judging from this ever present condition, these crabs seem to require more than ordinary concealment from the numerous foes which prey upon them, and hence this factor may be the determining cause for their association with medusæ in the present case. In this connection it is of interest to point out a certain degree of mutual adaptation on the part of the associating species. *Stomolophus* has a spacious and deep subumbrellar area, within which its large guest finds snug but ample accommodation; the marginal aperture allows ready ingress and egress; the manubrium is deeply grooved and pitted, enabling the crab to easily cling to its steed with great tenacity; and nematocysts are absent or so poorly developed as to be ineffective. The *Libinia,* on its part, is admirably shaped so as to conform to the contours of this peculiar residence, the back being strongly convex and the abdominal surface slightly concave; the legs are curved inward and end in sharply pointed tips; and finally, the mid-dorsal spines of the carapace are well developed and would assist in maintaining the position, especially when the crab was situated in the upper regions of the subumbrella, the site from which all of the nineteen specimens here recorded were dislodged.

The writer wishes to acknowledge the assistance of Dr. Waldo L. Schmitt of the United States National Museum, who has kindly provided references and identification checks, by Dr. Bigelow for the medusa and Dr. Rathbun for the crab, at a time when original sources were not otherwise available. Specimens illustrating this association are deposited in the United States National Museum and in the zoölogical collections of the University of South Carolina.

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REGENERATION IN A TROPICAL EARTHWORM *PERIONYX EXCAVATUS* E. PERR.

G. E. GATES.

In the course of a recent study of the *Oligochæta* of Rangoon a number of earthworms were found with reproductive apertures in normal arrangement and position on the segment but displaced one to four metameres anterior to or one to three metameres posterior to their usual position. The prostates and ovaries had the same definite relations to the sexual orifices in such individuals as in normal specimens and hence were anterior or posterior to their ordinary position to an amount equivalent to the dislocation of the reproductive pores. None of the various metameric and organ anomalies that appear in nearly all of the species of Rangoon earthworms were noticed in specimens of the type just mentioned.

Morgan (1895) found similar anomalies in specimens of *Allolobophora fœtida* and suggested (p. 404) that "In those cases in which openings of the vasa deferentia occur on a segment anterior to the 15th metamere,[1] we may be dealing with a case of incomplete regeneration of the anterior metameres. . . ." But further on he says, "That all of the cases can be explained in this way is, I think, highly improbable," and (p. 450) "regeneration of the anterior end will not account for any of those cases where the vasa deferentia open on a matemere posterior to the fifteenth."

No references to hypermeric regeneration of anterior ends in earthworms could be found in the literature available. The abnormality (posterior dislocation) was found so frequently, however, that a series of operations was begun with the idea of learning whether or not such anomalous specimens would develop as the result of regenerative processes experimentally induced in earthworms of the species concerned. One of the animals first used seemed to have such an unusual capacity for replacing lost parts that an extensive series of experiments was initiated to discover the limits of this capacity. After several months it became necessary to discontinue the work. Some of the data accumulated are of such interest, however, as to warrant publication in a preliminary note. At the first opportunity the experiments will be resumed.

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[1] In *A. fœtida* the normal position of the male pores is on segment fifteen.

In the preliminary series of operations worms belonging to nearly all of the species occurring in Rangoon were anæsthetized and the anterior ends excised at various levels. Only individuals of two species, *Pontoscolex corethrurus,* and *Perionyx excavatus* survived more than five days after the operation. At the end of two months only a small amount of segmentally undifferentiated tissue had been produced at the cut ends of worms of the first species, whereas all operated specimens of the second species had regenerated, in much shorter time, segmentally differentiated anterior ends. Further experiments were confined to this second worm.

Adult specimens of *P. excavatus* attain a length of 130 mm., and a diameter of 5 mm. The prostomium is large, fleshy, and characteristic. The intersegmental furrows are deep and clearly marked. Secondary annulation is lacking. The dorsal and lateral parietes are heavily pigmented. The clitellum is ring-shaped, yellowish or gray, contrasting sharply in color with the non-clitellar segments, and lies between the intersegmental furrows 12/13 and 17/18. The setæ are numerous and arranged in a closed ring around each segment behind the first metamere. The spermathecal apertures are large paired pores in the intersegmental furrows 7/8 and 8/9 (paired spermathecæ in viii and ix). The single female pore is clearly visible on xiv (paired ovaries in xiii). The larger male apertures are closely approximated mid-ventrally on xviii (paired prostates in xviii, paired seminal vesicles in xi and xii, paired naked testes in x and xi).

Mature worms can be secured in large numbers all the year round. They easily adjust themselves to the conditions of life in the laboratory, and have an exceedingly low mortality rate after operation or injury. The sharply delimited clitellum and large sexual apertures enable rapid determination of position, while the clear cut intersegmental furrows, the absence of secondary annulation, and the distinctly projecting setal circles, render segment counting simple, and the detection of metameric and setal anomalies easy. The dark red color of the normal segments sharply sets off the much lighter colored new tissue during the early weeks of regeneration and the presence of a protruding fleshy prostomium distinguishes a regenerating head from any other structure.

Only fully mature and normal worms were used. They were anæsthetized in a weak solution of chloretone and an excision was made by a clean cut, usually at an intersegmental furrow. The operated worms were kept in closed jars containing moist paper towelling. All results described in this paper were obtained in that part of the year known locally as the " cold season." The term is of course merely relative and indicates that the mercury is slightly lower all day long than at other seasons of the year. No effort was made to control the temperature but in the brick laboratory building the fluctuations of the mercury from midday to midnight are much less marked at this season of the year than out of doors.

In a short time after the operation a transparent conical outgrowth was visible at the cut end. In the case of regenerating anterior ends, oval fæcal pellets were found from the sixth day on, indicating that the digestive system had developed sufficiently in that short time to enable the worm to " bite " off, swallow, pass through the digestive system, and defecate pieces of the paper towelling. By the end of the second week the segmental differentiation of the regenerating heads was completed. Usually by the end of the third week the new segments contiguous to the old tail piece had attained the diameter of the metameres with which they were in contact, and were clearly setigerous. In the fourth week the pigment appeared in the regenerating segments.

Regeneration of Anterior Ends.

In all series of operations every surviving worm from which six or fewer segments had been amputated regenerated the exact number lost. If the prostomium or a fraction of the prostomium was removed a new prostomium or the missing fraction was replaced. In several worms a small wedge-shaped piece was removed dorsally, laterally, or ventrally from the anterior end of the stump. In each case such wedge-shaped pieces were replaced as well as the exact number of missing anterior segments. Only one animal died following operation anterior to segment six and in this single instance there was reason for suspecting that the death of the worm was not caused by the effect of the operation on the individual but by another factor to be discussed later.

When the excision was made at an intersegmental furrow posterior to 6/7 but anterior to 18/19 the ratio of the number of segments removed to the number regenerated, varied and the percentage of failure to survive the operation was higher. Here again there was reason for suspecting that many of the mortalities following operation were due to something more than the effect of the operation itself. None of the amputations at intersegmental furrows 10/11 or 14/15 resulted in hypomeric regeneration, but the number of worms involved (five at the first furrow and two at the second level) is too small to permit generalization. At all other intersegmental furrows between 6/7 and 18/19 hypomeric anterior ends have been formed. The largest number of segments not replaced is four and the smallest is one.

In one series four worms regenerated hypomeric heads each having one " half segment," *i.e.*, a metamere extending across the whole diameter of the animal but antero-posteriorly only about half the width of the segments immediately in front of and behind it. Such half segments were not setigerous and may represent incompletely differentiated metameres, or perhaps growth zones, although the specimens concerned were killed in the fourth week after the operation, by which time the segmental differentiation is usually completed. In only one regenerating anterior end was a wedge-shaped half-segment found and this was ventral in position.

Excisions at levels 7/8, 9/10, 10/11, and 13/14 alone resulted in hypermeric regeneration but the total number of such cases is too small for generalization. Only one extra segment was formed in each case. In these hypermeric worms all the regenerated segments except of course the first were setigerous and clearly outlined by intersegmental furrows. No half-segments, wedge-shaped or otherwise were found in heads of this type.

When the anterior end was excised posterior to 17/18 only hypomeric heads of ten to sixteen segments were formed. The number of worms operated on behind this level was too small to

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warrant a statement that total replacement of lost segments cannot take place posteriorly. It should be noted that when eighteen or more segments were amputated the digestive organs including part of the intestine which begins in the region of segment fifteen, as well as all of the reproductive organs were removed. When the cut was made between 17/18 and 24/25 twelve to sixteen segments usually regenerated. Posterior to 24/25 ten to fifteen segments regenerated. The posterior limit of anterior regeneration by tail pieces has not been determined, but it certainly lies unusually far back for earthworms, and at least in the last third of the length of the worm.

Table I.

Series Two.

Anterior Ends from One to Twenty Segments Removed.

85	worms were amputated.
81	worms survived operation.
48	worms regenerated exact number of segments lost.
27	worms regenerated a smaller number.
5	worms regenerated a larger number.
1	worm failed to regenerate.

Table I. summarizes briefly the results obtained from a characteristic series of operations. Table II. summarizes the regeneration in the region lying between segments six and eighteen

Table II.

Series Two.

Results of Operations between Intersegmental Furrows 5/6 and 17/18.

Number of Segments Removed.	Number of Specimens Operated.	Worms with Regeneration of Exact Number of Segments Lost.	Worms with Hypomeric Heads.	Worms with Hypermeric Heads.	Worms without Regeneration.
7	7	4	2	1	—
8	8	5	3	—	—
9	10	5	3	2	—
10	5	4	—	1	—
11	2	1	1	—	—
12	5	1	3	—	1
13	4	2	1	1	—
14	2	2	—	—	—
15	4	—	4	—	—
16	3	2	1	—	—
17	4	2	2	—	—

from the second series of operations. In Table III. is a condensed statement of hypomeric and hypermeric regeneration in the same region but including results of more than one series of operations.

Table III.

Hypomeric and Hypermeric Anterior Ends Regenerated at Cuts between Segments Six and Eighteen.

Number of Segments Removed.	Number of Segments Regenerated.
7	3, 6, 8
8	6, 7
9	6, 8, 10
10	11
11	8, 10
12	8, 10
13	11, 14
14	— —
15	11, 12, 13
16	14
17	13, 14, 15

Regeneration of Posterior Ends.

No special attempt was made to study tail regeneration by anterior pieces. Numbers of amputated anterior portions were kept in conditions similar to those of the regenerating tail pieces and the daily records of the experiments contain some notes on these amputated pieces. Anterior ends of more than twenty segments may regenerate new tails. No information is available as to tail regeneration anterior to segment twenty. Amputation at various levels posterior to 20/21 usually resulted in rapid formation of new posterior ends. None of these regenerating anterior portions were kept alive longer than four weeks so that it is not possible to make any positive statement as to the ratio of segments replaced to those lost. There is no reason to suspect, however, that anterior portions of twenty segments or more cannot replace all segments especially if provided with food.

Mutilations.

While the first series of experiments was under way a collection of worms containing several regenerating specimens of *P. excavatus* was brought into the laboratory. Arrangements were at once made to secure several thousand worms of this species from various quarters of the town. A considerable number of individuals thus obtained had evidently been deprived in some manner of a head or tail or quite rarely of both ends. One collection of more than three hundred worms contained more than a hundred mutilated specimens. This percentage was so high that previous digging was suspected of being the cause of the mutilations. As the collections had been made on successive days over a period of several weeks, it is possible that some at least of the mutilated specimens were produced in this way. In order to avoid this factor, collections were made at several localities which presented every appearance of having been undisturbed for months. Such collections also contained high percentages of mutilated individuals. Practically all the mutilations found were amputations of a head or a tail at an intersegmental furrow. Only three regenerating individuals were found in which excision had occurred in the middle of a segment. In these specimens the missing half segment had been regenerated as well as a portion of the tail behind. One worm mentioned elsewhere had been deprived of dorsal portions of two segments in addition to the anterior end.

Table IV. summarizes the information available from records of the collections. It should be noted that all mutilations included within this table had been produced at least several days previous to the time of collection. Such few specimens as were brought into the laboratory obviously injured as the result of collecting processes were, of course, discarded and not included in the tables.

Through such collections more than fifteen worms were secured that had lost their heads. Nine were regenerating new anterior ends when brought into the laboratory. Of this latter number four were either immature or if mature had lost more than eighteen segments, for there were no characteristic sexual markings to make a determination possible. The remaining five specimens had lost their heads anterior to the prostatic segment. By assuming that the prostatic segment of each of these animals was the eighteenth metamere before the mutilation, as in normal worms, it was possible to determine the number of segments lost and the type of regeneration that ensued. On the basis of this assumption three individuals had regenerated hypomeric anterior ends, one had produced a hypermeric anterior end and the others had exactly replaced the number of segments lost. The single worm with hypermeric regeneration had lost the first thirteen segments as well as dorsal portions of segments xiv and xv and had not only replaced the lost dorsal portions but had also formed *fifteen* perfectly normal and clearly outlined setigerous segments and the non-setigerous prostomial segment.

Table IV.

Collection Number 1.

Normal worms	231
Mutilated worms	104
Worms with tail mutilation	98
Worms with head mutilation	6
Worms regenerating a tail	77
Worms regenerating a head	3

Collection Number 2.

Normal worms	301
Mutilated worms	87
Worms with tail mutilation	84
Worms with head mutilation	3
Worms regenerating a tail	71
Worms regenerating a head	2

Collection Number 3.

Normal worms	1
Mutilated worms	6
Worms with tail mutilation	5
Worms with head mutilation	1
Worms regenerating a tail	3
Worms regenerating a head	1

Collection Number 4.

Normal worms	?
Mutilated worms	?
Worms regenerating a tail	49
Worms regenerating a head	4

It is evident therefore that both in its natural environment and under experimental conditions in the laboratory *P. excavatus* may regenerate hypermeric anterior ends. In view of this demonstration there seems to be no need for further search, at least for the present, for other explanation of the origin of the anomalous specimens mentioned at the outset of this paper. Such abnormalities at least in the species under discussion appear to be adequately accounted for as the products of hypo- or hyper-meric regeneration.

VARIA.

Additional extensive experiments were begun to obtain information as to the posterior limit of anterior regeneration by tail portions, the anterior limit of posterior regeneration by head portions, the regeneration of pieces with two-cut ends from various regions of the body, etc. These experiments failed completely, as far as the objects in view were concerned, because of a constant series of accidents which will be described under the title of autotomy. A few notes from the records of these experiments are given herewith to indicate more clearly the unusual regenerative characteristics of this worm.

A. A short piece of eight segments from the middle portion of worm 109 was still living on the eighth day after the operation and responded quickly to various sorts of stimuli. Both ends had healed over without any signs of regeneration.

B. The forty-one anterior segments of worm 110 regenerated in nine days new tissue five and one half millimeters in length.[1] The new tail was composed of a long, metamerically differentiated portion containing more than thirty segments, the anal segment, and between these two a short region of formation of new metameres.

C. The anterior end of worm 132, a piece fifteen millimeters in length, composed of twenty-four segments regenerated in two weeks a tail fifteen millimeters long containing in the segmentally differentiated region, fifty-four segments.

D. A shorter anterior end from another worm regenerated at its posterior cut surface a *head* of several segments with a characteristic mouth and prostomium.

E. A twenty-three metamere fragment thirteen millimeters in length from the posterior half of worm 118 in two weeks regen-

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[1] The measurements noted were made on material that had been killed by dropping into strong methylated spirits and then hardened in formalin. Old and new tissue appeared to be uniformly contracted by this mode of killing and fixing.

erated at one end a new head containing thirteen segments and a prostomium and at the other end a tail two and one-half millimeters long, containing in addition to the anal segment and the growing region twelve differentiated segments.

F. A shorter portion from the posterior half of another worm regenerated at one end a head and at the other end a single anal segment.

G. A short tail fragment regenerated at its injured anterior end a structure exactly similar in appearance to the tail developed at the cut posterior surface of an anterior piece.

H. The nine anterior segments containing both pairs of spermathecæ were removed from worm 82. Eight segments regenerated. Characteristic spermathecal pores appeared in intersegmental furrows 6/7 and 8/9 (the posterior pair of pores being located between the last of the old and the first of the new segments!).

I. Several other worms from which anterior ends containing one or both pairs of spermathecæ had been removed regenerated heads with one or two pairs of spermathecal pores at various intersegmental levels. These worms were killed three weeks after the operation, hardened in formalin and dissected. Definitive spermathecæ had not been formed by that time. The site of each spermathecal pore was marked internally by the presence of a lump of soft spongy tissue. Some of these specimens with regenerated spermathecal pores were very similar to specimens of this species secured by Beddard (1886) from the Philippines. It is quite possible if not probable that many or even all of the thirteen anomalies described and figured by Beddard as "variations" were the result of regenerative processes.

J. Seventeen anterior segments were removed from worm 149. When the animal was killed at the end of the fourth week after the operation, seventeen segments had been regenerated. In the usual position on segment fourteen was a typical female pore. The clitellar segments (xiii–xvii) were distinctly lighter in color than the other new segments, indicating the beginning of clitellar differentiation. Although the head was carefully fixed and hardened the tissues were too soft and spongy internally for dissection and no reproductive organs could be demonstrated.

Autotomy.

Several references have already been made to disturbing factors which interfered with the success of some of the experiments. One of factors, the most important, was a tendency for the worms to break into fragments in early hours after the operation. For want of a better term this process of fragmentation will be referred to as autotomy. Only very rarely was this fragmentation observed to occur later than the first twenty four hours after the operation, and then only very small portions usually consisting of one or two segments were thrown off.

In the first series of operations on *P. excavatus* many of the operated worms autotomized one or more pieces from the posterior portion. In another series of anterior operations thirty two out of forty two animals autotomized portions of the tail ranging from seven to sixty millimeters in length. No series of operations in which tail portions of the worm were watched was free from this tendency to fragment. In the majority of cases one or two short pieces were autotomized from the posterior end of the major operated portion. Such fragments were usually dead when discovered but very often lacked the pungent odor so characteristic of decaying earthworm. Occasionally the fragmentation was much more striking and extensive. Worm *B* 9 from which eleven anterior segments had been removed broke into pieces. Worm *D* 19 from which *X* anterior segments had been removed broke entirely into pieces six to ten millimeters in length. Several other specimens from which eight or nine segments had been removed also autotomized extensively.

Autotomy is usually understood to be a throwing off by the animal of a small portion which usually dies without producing a new animal but in *P. excavatus* apparently any fragment from any region may survive, or more than one of the fragments may survive, with the survival determined by the presence or absence of something in the worm and not by the position of the fragment along the axis of the animal. The autotomy was observed only in posterior portions. The length of the tail however was of no significance. Posterior portions from one third to approximately nine-tenths of the length of the original worm autotomized extensively while anterior portions longer than one-half never autotomized. Several posterior portions about equal to one-half the length of the original worm autotomized from each end one or two pieces which died while the longer middle portion survived.

The experiments were discontinued before a thorough analysis of various aspects of this interesting tendency to fragment could be completed. Two experiments, however, provided a hint, not only as to the cause of the fragmentation but also as to the cause of certain other disturbing factors. Worm 94 was anæsthetized as usual and cut into three approximately equal portions, each of which was kept separately in a tightly closed jar. No autotomy occurred in any of the jars. The head piece survived and regenerated a tail. The middle piece regenerated at one end an anal segment and at the other end a head about three millimeters in length composed of fifteen segments. On the day following the operation the tail portion was collapsed and flattened, dead, but without noticeable odor of decay. In the jar were three flies which must have been present in the tail portion of the worm at the time of the operation, as the jar was not opened until after the flies had appeared. Through the kindness of entomologists at the Imperial Bureau of Entomology, London, these flies have been identified as *Aphiochæta scalaris* Lw.

A number of head portions ranging from twenty-five segments to about half the length of the worm were kept in a single, large, tightly-closed jar. A few portions died during the first three days after the operations and were removed. At the end of the week four of the head pieces were still living and apparently in good health although without signs of regeneration. The only traces to be found of the other head portions were numerous tubular fragments of transparent cuticle. Crawling around inside the jar were numerous small insect larvæ. When the jar was opened two small flies very similar in appearance to those from the other worm escaped. There seems to be no reason for doubting that some larval stage of the fly was parasitic in these worms at the time of the operation and it is at least possible that the presence of parasitic fly larvæ is the factor responsible for the autotomy as well as other disturbances in the operated worms. It should be noted, however, that *A. scalaris* is a very general feeder and that it has been bred from all sorts of decaying matter. The Director of the Imperial Bureau of Entomology writes: "I should be inclined to suppose that the attack on the earthworms that you have noticed was accidental, for it seems unlikely that this fly would prove to be a true parasite."

SUMMARY.

1. *P. excavatus,* an earthworm occurring in large numbers in dung heaps and soil rich in decomposing organic matter in Rangoon has a regenerative capacity very much higher than any known at present from megadrilous Oligochæta with the single exception of the limnic *Criodrilus lacuum* Hoffm., from Europe. The rate at which regeneration is completed is rapid.

2. Posterior portions can replace the anterior segments lost if the number of metameres removed is seventeen or less. When more than seventeen segments are removed only ten to fifteen metameres were regenerated.

3. The posterior limit of head regeneration lies somewhere in the last third of the length of the worm.

4. Spermathecal apertures and female reproductive pores may develop on regenerating anterior ends.

5. Anterior pieces of twenty segments or more may regenerate tails.

6. A heteromorphic head may be regenerated at the posterior end of a very short anterior piece.

7. A heteromorphic tail may be regenerated at the anterior end of a very short tail piece.

8. A piece of twenty or more segments from the middle of the worm may regenerate at one end a tail and at the other end a head.

9. Regenerated heads may be normal, hypomeric, or hypermeric. Hypomeric and hypermeric regeneration is considered an adequate explanation of the origin of abnormalities described as anterior or posterior dislocation of the reproductive organs.

10. In collections made in various quarters of the town a high percentage of the individuals secured had been mutilated by the amputation of a head, a tail, or both. Many of the mutilated specimens were regenerating the lost parts when collected.

11. One or more pieces both anteriorly and posteriorly are very frequently autotomized after operation by posterior portions. Sometimes the whole tail portion fragments into pieces six to ten millimeters in length. Anterior ends have not been observed to autotomize.

12. A fly *A. scalaris* Lw. has been bred from portions of *P. excavatus.* Parasitism by this insect may possibly be responsible for the mutilated specimens and for the autotomy following operation.

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STUDIES ON THE PHYSIOLOGICAL EFFECTS OF HYDROGEN CYANIDE.

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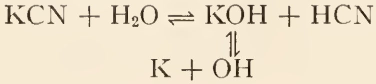
The question of the physiological effects of cyanide has been of considerable interest since Claude Bernard (1) in 1857 noticed that the venous blood of vertebrates is bright red after treatment with cyanide. It is well known that cyanides in dilute solutions act in general as protoplasmic depressants. In most cases this depressing effect can be attributed to the inhibition of oxidations. Investigations by Allen (2), Child (3), Hyman (4, 4*a*, 4*b*), Vernon (5), Buchanan (6) and others show that potassium cyanide, even in extremely dilute solutions, depresses reversibly the rate of respiration in *Planaria*. Lund (7), however, noted no decrease in oxygen consumption in *Paramecia* placed in potassium cyanide solutions. The fact that dilute solutions of cyanide act as anesthetics is equally well known (Heilbrunn, 8; Osterhout, 9). An important difference between the effects of a typical anesthetic, such as ether, and cyanide was pointed out by Heilbrunn (8) who showed that ether decreases the viscosity of protoplasm while KCN in anesthetic concentrations increases it. Heilbrunn, therefore, concluded that in the case of sea urchin eggs there are two types of anesthesia; in one the viscosity of the cytoplasm is decreased and in the other it is increased. The toxic action of cyanide in concentrated solutions is also well established (Hyman, 4). In vertebrates the toxicity of cyanide seems to be due to its effect on the central nervous system, as shown by Geppert (10) and Dontas (11). Child (12) showed that the portion of an organism with the highest rate of metabolism is most susceptible to cyanide, and that young organisms having a high rate of metabolism are more susceptible than adults with lower rates. For a detailed review of the literature on the various phases of the cyanide problem, the reader is referred to the paper of Hyman (4).

The question of the effect of cyanide on the permeability of membranes is a debatable one. It is generally considered that anesthetics, such as alcohol and ether, decrease permeability (Lillie, 13; Lullies, 14; McClenden, 15; Osterhout 9*a*). Wertheimer (16), on the other hand, concluded that narcotics increase the permeability of frog skin while Krehan (17) showed that KCN increases the permeability of plant cells to many substances.

Most workers with cyanide have used potassium cyanide, which in an aqueous solution is strongly alkaline, due to the manner in which it dissociates:



$$\mathrm{KCN} + \mathrm{H_2O} \rightleftharpoons \underset{\underset{\mathrm{K} + \mathrm{OH}}{\upharpoonleft\downharpoonright}}{\mathrm{KOH}} + \mathrm{HCN}$$

Hydrogen cyanide in an aqueous solution acts as an extremely weak acid, dissociating only to a slight degree. In view of the fact that the question of the effect of cyanide on permeability is a debatable one and since most of the previous workers have used KCN, it was thought advisable to study in detail the penetration of hydrogen cyanide through living membranes as well as its effect on the membrane.

The investigations were conducted at the Zoölogical Laboratory, University of Pennsylvania, for which privilege the writer wishes to acknowledge his indebtedness to Doctor C. E. McClung. The writer is also under obligations to Doctor J. H. Bodine, under whose direction the investigations were conducted, for many helpful suggestions throughout the progress of the work.

It was found convenient in this work to use the artificial "cell" devised by Jacobs (18) and constructed in the following manner: a hard glass tube 7 cm. long and 1.5 cm. in diameter was tapered at one end to an opening of one cm. in diameter and the tapered end provided with a tip. The skin from the hind legs or back of a freshly killed frog (*Rana catesbiana* or *R. pipiens*) was carefully stretched over the lipped end of the tube and held in place by a rubber band. The skin was so placed over the tube that the inside, or flesh side, of the skin was exposed to the exterior. The "cell" so constructed was placed in a 100 cc. quinine bottle and both the "cell" and bottle fitted with rubber stoppers. The inside of the "cell" was filled with a borax-boric acid buffer solution and a solution of HCN in a borax buffer was placed in the quinine bottle. It was necessary to use a buffer which would not injure the skin or react chemically with either the cyanide or the silver nitrate solution used to determine the concentration of cyanide. The pH values from 6.8 to 9.2 were obtained by changing the relative amounts of sodium borate and boric acid; for pH values below 6.8 it was necessary to add small amounts of nitric acid. The addition of HCN did not change the pH of the solution of either the borax or the borax plus nitric acid. Pure liquid hydrogen cyanide was used. No leakage occurred around the skin as shown by the fact that when a "cell" was filled with an

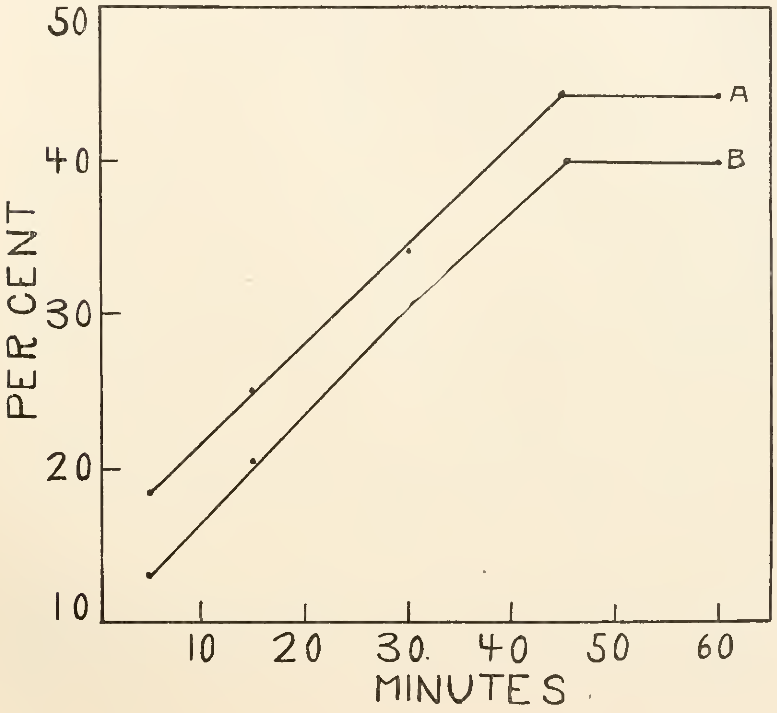


Fig. 1. Curve showing the relation of the position of the skin to the permeability of the skin to cyanide. *A*, skin with the flesh side out; *B*, skin normal, flesh side in. Abscissæ, time in minutes; ordinates per cent. cyanide.

indicator and placed in a dilute solution of HCl the indicator did not change color. Several tests were conducted by reversing the skin and it was noted that the membrane was slightly more permeable to HCN when the skin was turned inside out than when it was in a normal position. The difference, however, was so slight that it is not significant (Fig. 1).

The "cells" after being filled with a borax buffer and put in a solution of HCN, were placed in a water bath at a constant temperature of 25° C. for one hour. At the end of that time equilibrium was reached between the cyanide inside and outside the cell. Five cubic centimeters of the internal and external solution were titrated with $N/50$ silver nitrate, using a one cc. pipette as a burette. The concentration of the cyanide solution used was $M/313$. That the skin was not killed at this concentration was easily demonstrated by substituting for the cyanide solution mineral acids, known not to penetrate living membranes. No change in intracellular acidity was noted in such control experiments. Experiments were conducted using external and internal solutions of various pH values, the external pH varied from 5. to 8.6 and the internal pH from 6.5 to 8.0. The results plotted in Fig. 2, show the relation of concentration of the total cyanide (HCN and CN) found in the cell at equilibrium to the various external and internal pH values. From this figure it may be noted that the penetration curve closely approximates the dissociation curve (x) and that the total concentration of cyanide inside the cell corresponds very closely to the undissociated cyanide in the external solution. The degree of dissociation represented in the curve was calculated from the formula

$$\log 1/H = \mathrm{pH} = \log 1/K + \log a/1-a \quad (19),$$

where $a =$ degree of ionization: $K =$ dissociation constant, for $\mathrm{HCN} = 4.7 \times 10^{-7}$ (20).

Figure 2 shows that at equilibrium, when the pH is from 5. to 5.5, the concentration of total cyanide within the cell is equal to the amount of cyanide in the external solution. From the dissociation curve it is evident that at this pH there is practically no dissociation, all of the cyanide being in the molecular condition. As the external pH was increased, the penetration curve rapidly dropped. Likewise as the pH was increased the degree of ionization was increased. At a pH of 7.0 the dissociation is practically 50 per cent. and the concentration of cyanide in the cell is about 40 per cent. of the concentration of the external solution.

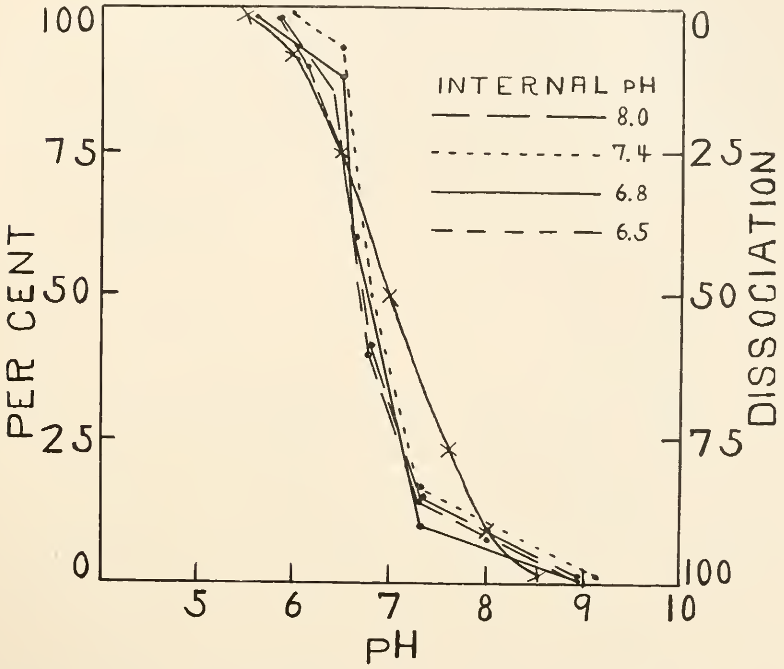


Fig. 2. Curve showing the effect of pH on the permeability of frog skin to HCN. Abscissæ represents the external pH; ordinates the per cent. of total cyanide on the right and the degree of ionization on the left. Calculated degree of dissociations represented by *X*. Internal pH values indicated as follows: short dashes 6.5, solid line 6.8, dotted line 7.4, long dashes 8.0.

At a pH of 9.0 dissociation is practically complete and accordingly little or no cyanide is present in the cell. It is evident that hydrogen cyanide seems to penetrate frog skin chiefly in the form of molecules and not as ions. It is also apparent from Fig. 2 that the intracellular pH varying from 6.5 to 8.0 does not affect the permeability of frog skin to hydrogen cyanide. Brooks (21) stated that changing the pH of the sap of Valonia with CO_2 and NH_3, changed the amount of 2—6—dibromo phenol indophenol that entered the cells. However, she states and also Scarth (22) shows that the pH of the sap in the vacuole could be no criterion of the pH of the protoplasm.

Experiments with Tadpoles, Daphnia and Elodea.

Experiments conducted with young bull frog tadpoles (Fig. 3) and *Daphnia* (Fig. 4) gave the same relative results as the experiments conducted with cyanide on frog skin "cells." After being separated from their cultural medium by cheese cloth, the organisms were placed in Syracuse watch glasses filled with a solution of HCN in a borax buffer solution. It was previously determined that the animals were not killed in the buffer solution free of cyanide until after an exposure of three to four hours. On account of the anesthetic action of the cyanide, tadpoles did not prove to be good material for this work, since it was difficult to determine the time of death. When *Daphnia* was used the beat of the heart could be observed under a binocular microscope and the death point determined at the instant the heart stopped beating. It was noted, that the heart continued beating for some time after

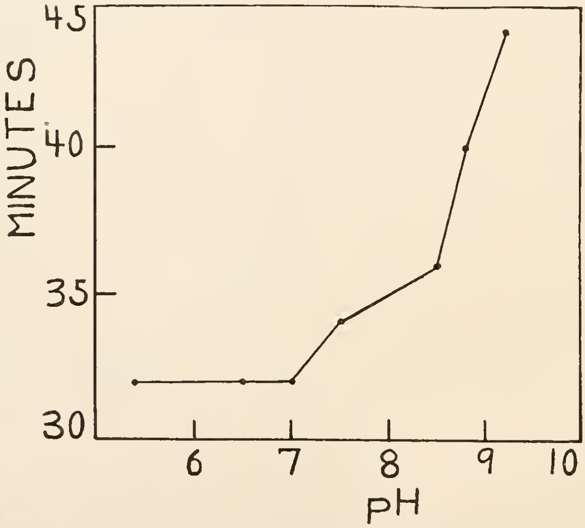


Fig. 3. Curve showing the effect of pH on the toxicity of HCN to young bullfrog tadpoles. Abscissæ represents the pH; ordinates the time in minutes required to produce death. Ten animals were used in each test.

other body movements had ceased and that the organism did not recover after the heart stopped.

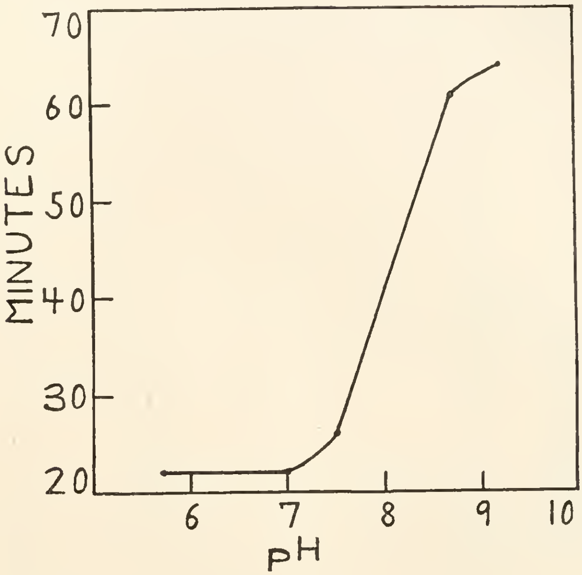


FIG. 4. Curve showing the effect of pH on the toxicity of HCN to *Daphnia*. Abscissæ represents pH; ordinates time in minutes required to kill 95 per cent. of the organisms.

It is obvious from Fig. 3 and 4 that less time was necessary to kill the animals in acid solution than in the alkaline solution. It required twenty-two minutes to kill *Daphnia* at a pH of 5.7 to 7.0; forty minutes at a pH of 8.0 and sixty-four minutes at a pH of 9.0. The concentration of cyanide used in the above experiment was *M*/450. The results indicated what would be expected from the study of the frog skin "cell," where more cyanide entered when the external pH was acid than when alkaline. The animals were killed first in solutions of the same pH values in which HCN penetrated the frog skin cells most quickly.

The effect of the pH on penetration of cyanide was further checked by studying its effect on the streaming of protoplasm in *Elodea* cells (in press). The streaming of the protoplasm can be observed under the high power of a microscope while the cells are immersed in a solution of cyanide. The pH was controlled, as before, by a borax buffer. Leaves of *Elodea* near the growing tip of the branch were placed in a solution of hydrogen cyanide in Syracuse watch glasses and the time noted when the streaming of the protoplasm ceased. The results of a typical set of experiments are plotted in Fig. 5. Again the results show that the streaming of protoplasm stopped first in solutions of the same pH values in which HCN penetrated the artificial frog skin "cell" most quickly. Thus, in determining the toxicity of hydrogen cyanide to any organism, the value of controlling the hydrogen ion concentration of the solution is apparent.

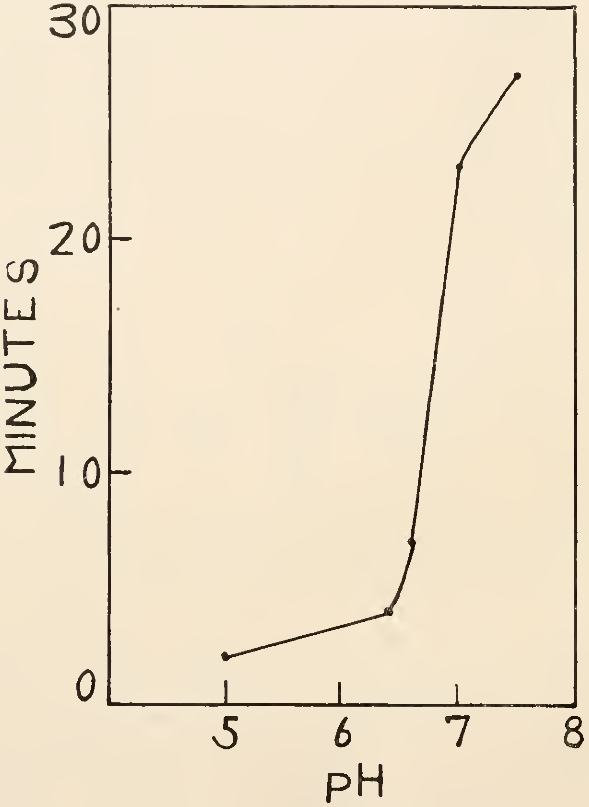


Fig. 5. Curve showing the effect of pH on the streaming of protoplasm in *Elodea* cells. Abscissæ represents pH; ordinates time in minutes required for streaming to stop.

It is obvious from the foregoing discussion that hydrogen cyanide penetrates living membranes chiefly in the form of molecules and in this respect is similar to other weak acids. Jacobs (18*a*) showed that carbonic acid killed various species of protozoa in a different order than mineral acids, which act primarily through their H ion (Collett, 23). This indicated to Jacobs, that the physiological effect of CO_2 was due to the entrance of the molecule. Beerman (24) and Bodine (25) obtained similar results with H_2S and HCN respectively. More recently, Osterhout (9*b*) and Osterhout and Dorcas (9*c*) showed by direct analysis that hydrogen sulfide and carbonic acid penetrated the living cells of *Valonia* chiefly in the form of molecules and not as ions. Brooks (21*a*) found that the amount of 2 — 6 — dibromo phenol indophenol in the sap of *Valonia* was proportional to the amount of undissociated dye in the external solution.

EFFECT OF TEMPERATURE.

A series of experiments was conducted to determine the effect of temperature on the permeability of frog skin to hydrogen cyanide. The results are plotted in Fig. 6. It is evident from these curves that the higher the temperature the greater is the concentration of intracellular cyanide at any stated time. The curve suggests that of a typical unimolecular reaction as can be easily demonstrated by calculating the velocity constant from the following equation:

$K = 1/t \log a/a{-}x$ (26) in which $x =$ the amount of cyanide in the cell at any time t; $a =$ the amount of cyanide in the cell at equilibrium (Table I.).

TABLE I.

VELOCITY CONSTANT K CALCULATED FROM THE UNIMOLECULAR EQUATION $K = \frac{1}{t} \log \frac{a}{a-x}$ AT VARIOUS TEMPERATURES.

External and internal pH 6.8.

c°.				16°				25°.				0°.				34.5°.			
t	*a*	*x*	*k*	*t*	*a*	*x*	*k*	*t*	*a*	*x*	*k*	*t*	*a*	*x*	*k*	*t*	*a*	*x*	*k*
Min.																			
15	1.84	.99	.0222	15	2.37	1.18	.0207	15	3.56	2.24	.0276	15	3.63	1.84	.0204	15	3.69	2.04	.0251
30		.158	.0256	30		2.17	.0357	30		3.11	.0252	30		2.97	.0256	30		2.90	.0223

It will be noted from Table I. that the velocity constant for any temperature is fairly consistent with the exception of 16°, and that K for the various temperatures is nearly constant. Plotting the log of the rate against the reciprocal of the absolute temperature, a curve was obtained as indicated in Fig. 7. The rate represents the time when the intracellular concentration of cyanide is twenty-five per cent. of the external concentration. It will be seen from this figure that there is a break in the line at 16° C. (.003415). Calculating Q from the Vant Hoff-Arhenius equation, (26)

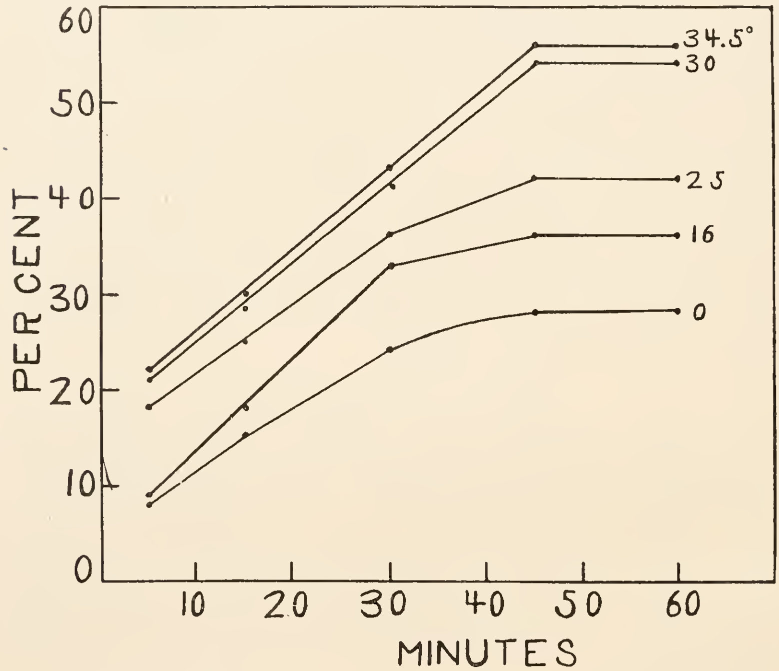


Fig. 6. Curve showing effect of temperature on the permeability of frog skin to HCN. Internal and external pH 6.8. Temperatures used were 0°, 16°, 25°, 30°, 34.5° C. Abscissæ represents exposure in minutes; ordinates per cent. of total cyanide.

$$K_2/K_1 = Q/2\left(\frac{T_2 - T_1}{T \cdot T_2}\right),$$

a value of 11,179 is obtained at a temperature from 16° C. to 34.5 and 4,300 for 0° to 16°.

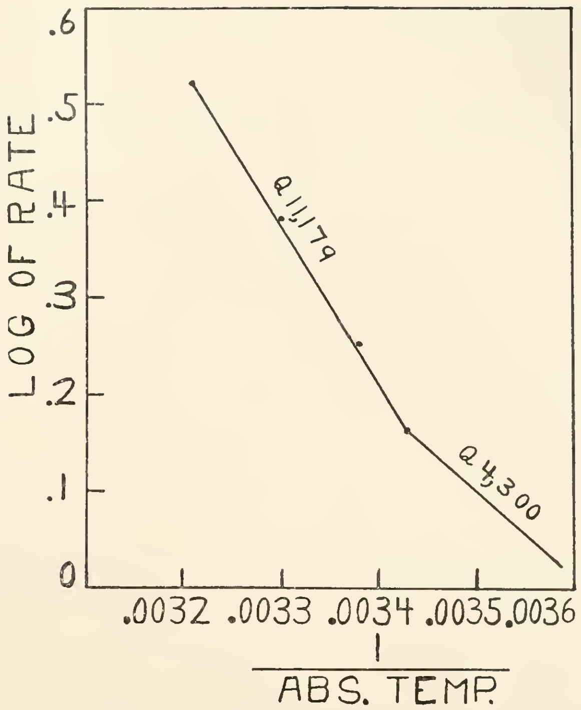


FIG. 7. Curve showing the log of the rate plotted against the reciprocal of the absolute temperature. Rate calculated from Fig. 5 as the time taken for the intracellular cyanide to equal 25 per cent. of the external concentration. Calculated from the formula

$$\frac{K_2}{K_1} = \frac{Q}{2}\left(\frac{t_2 - t_1}{t_2 \cdot t_1}\right).$$

Abscissæ represents reciprocal of the absolute temperature. Ordinates, the log of the rate.

RELATION OF CONCENTRATION.

Experiments were undertaken to determine the effect of the concentration of cyanide in the external solution on the penetration of hydrogen cyanide through frog skin. The external and internal pH and the temperature were maintained constant at 6.8 and 25° C. respectively. The concentrations of cyanide were *M*/109, *M*/124, *M*/160, *M*/196, *M*/225 and *M*/313. The results of such an experiment are plotted in Fig. 8. Each point on the curve represents the average of three to five tests. It may be seen from this figure that, with the exception of the two low concentrations ($M/225$ and $M/313$), the total amount of cyanide within the cell at equilibrium was the same for all the concentrations. The rate of entrance of cyanide increased with an increase in concentration. At a concentration of $M/109$, equilibrium was reached within 30 minutes, while at lower concentrations equilibrium was not reached for a period of 45 minutes. The fact that the skin is not killed can be proved by substituting for the cyanide solution a mineral acid, which is known not to pass through living membranes, and testing the pH of the internal solution. As there is no change in the intracellular acidity, it is evident that no acid has passed through the skin.

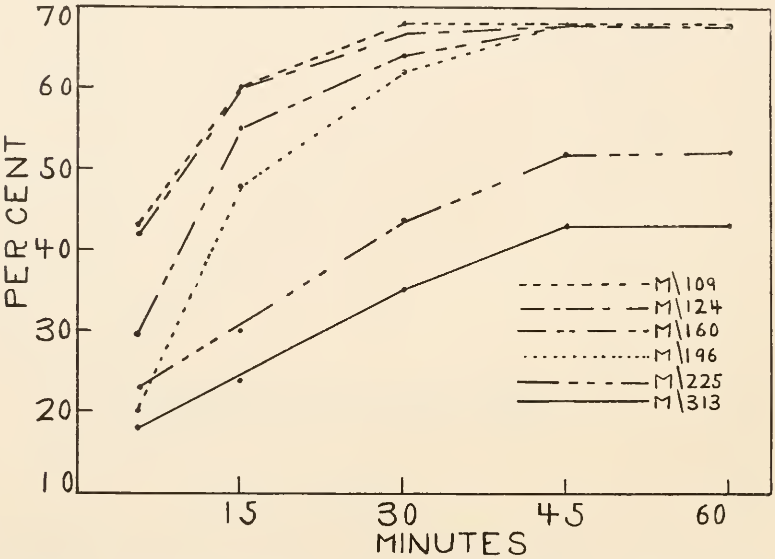


FIG. 8. Curve showing the effect of concentration of HCN upon the permeability of frog skin to cyanide. Concentrations of HCN used were $M/109$, $M/124$, $M/160$, $M/196$, $M/225$, and $M/313$. Abscissæ represents the time in minutes; ordinates per cent. cyanide.

Although, it is known that the frog skin is not killed by the above treatment with HCN, it is desirable to ascertain what effect the cyanide does have upon it. A series of experiments was conducted to determine the effect of hydrogen cyanide on the potential difference of frog skin and the results are given below in detail.

Effect of Hydrogen Cyanide on the Potential Difference of Frog Skin.

Osterhout (9*c*) and others have shown that the electrical resistance of an organism is an excellent indicator of its vitality and that death is accompanied by an increase in permeability. An increase in permeability is equivalent to an increase in the electrical conductivity or to a decrease in resistance. In order to determine the physiological effect of hydrogen cyanide on frog skin, it was thought advisable to study its potential difference before and after being immersed in various solutions of this chemical. The apparatus was similar to that previously used in the first part of this paper with the exception that non-polarizable zinc electrodes were placed inside and outside the cell and the potential read on a potentiometer. The same solution was placed inside and outside the cell. The skin from the hind legs of bull frogs (*Rana cates-*

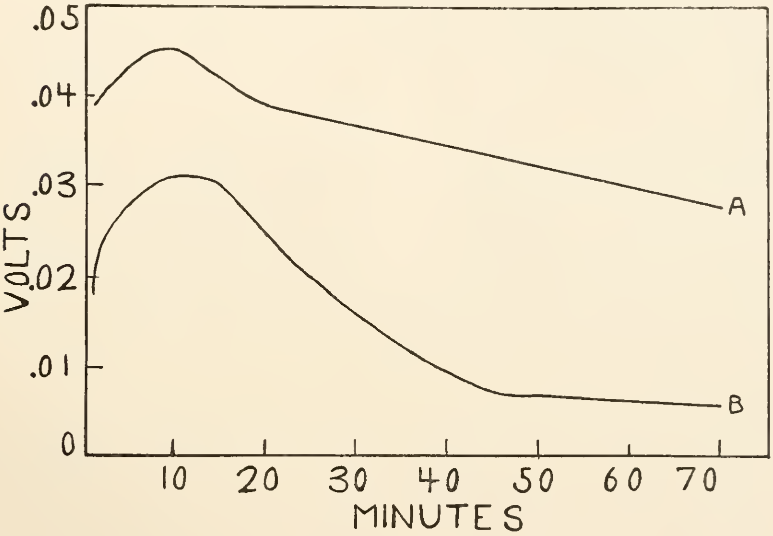


Fig. 9. Control experiments; curve showing the P.D. of frog skin in *A*, Ringer's solution and *B*, borax-boric acid buffer. Abscissæ represents the exposure in minutes. Ordinates P.D. in volts. Different pieces of skin from the same frog were used.

biana) was used. Since the potential difference of the skin from different frogs and also different pieces of skin from the same frog, varied considerably, it was necessary to repeat each experiment many times and only characteristic curves of single experiments will be given. There is some evidence at hand to indicate that the conditions under which the frogs are kept influences to some extent the potential difference of the skin. It was observed in several instances, when the temperature of the vivarium dropped several degrees below normal, that the potential difference of the skin also dropped and that on warm days the potential difference was usually higher than that at other times.

Control experiments were conducted by placing the skin in solutions of borax-boric acid buffer and Ringer's solution. The result of a typical control test is given in Fig. 9. Readings on the potentiometer were taken every minute. The temperature varied during the tests from 20–21° C. The experiments were run in parallel series using different pieces of skin from the same animal. It will be noted from Fig. 9, that there was an initial rise in P.D. followed by a gradual decline. The two curves are approximately parallel indicating that the borax buffer is no more toxic to frog skin than is the Ringer's solution. The pH of the Ringer's solution was about 8.2 and the borax 6.8.

A series of experiments was undertaken to determine the relation of the concentration of cyanide to the potential difference of frog skin. The cyanide solution was made by adding pure liquid HCN to a borax buffer at a pH of 6.8. The concentrations of cyanide used were $M/136$, $M/154$ and $M/225$. Fig. 10 shows the results of a typical set of experiments. The cells were placed in a borax buffer for ten minutes, then removed and placed in the cyanide solution. The skin was allowed to remain in the cyanide solution for various periods of time, then removed and placed in a borax buffer free from HCN. The period between arrows indicates the time that the cells were exposed to cyanide. In all cases it may be observed that the skin completely recovered after being removed from the cyanide solution. After the cells had been removed from the borax solution and placed in cyanide solution at a concentration of $M/225$, a great stimulation occurred, followed by a gradual drop in the potential difference to the base line. This stimulation, characterized by a rise in potential difference, is also evident at a concentration of $M/154$ but not nearly to the same degree as with the weaker concentration of cyanide. The drop following stimulation at a concentration of $M/154$ is practically of the same magnitude as obtained with a $M/225$ solution. In the case of the $M/136$ solution there was no stimulation but the potential difference dropped suddenly to the base line. The skin

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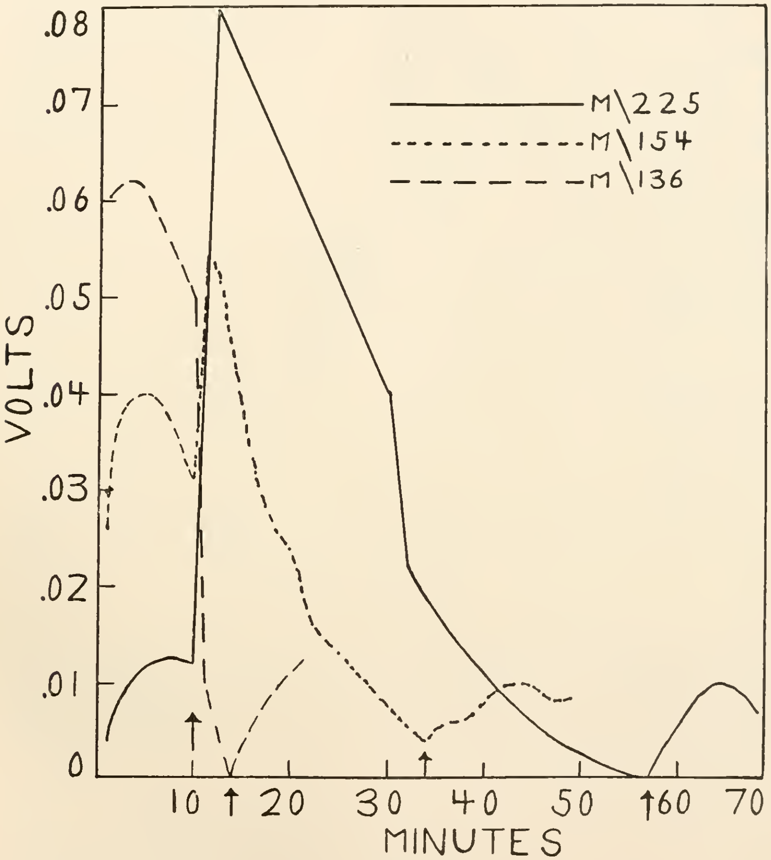

Fig. 10. Curve showing the relation of concentration of HCN to the P.D. of frog skin. Concentration of total cyanide $= M/225$, $M/154$ and $M/136$. Same solution on both sides of the membrane. The period between arrows indicates the time that the skin was exposed to cyanide, at other times the skin was exposed to borax buffer free from HCN. Abscissæ represents the time in minutes; ordinates the P.D. in volts. Different pieces of skin from the same frog were used.

recovered when removed from the cyanide. It is obvious from Fig. 10, that a weak solution of cyanide acted at first as a stimulant and this was followed by a delayed toxicity. As the concentration of cyanide was increased, the stimulation decreased and the toxic effect increased until a point was reached where stimulation no longer occurred and at this point the toxic action was pronounced. This initial stimulation of frog skin by cyanide was apparently overlooked by Lund (*7a*) who took readings every fifteen minutes. He did notice, however, a rapid rise followed by a rapid fall in the electrical resistance in Obelia during a period

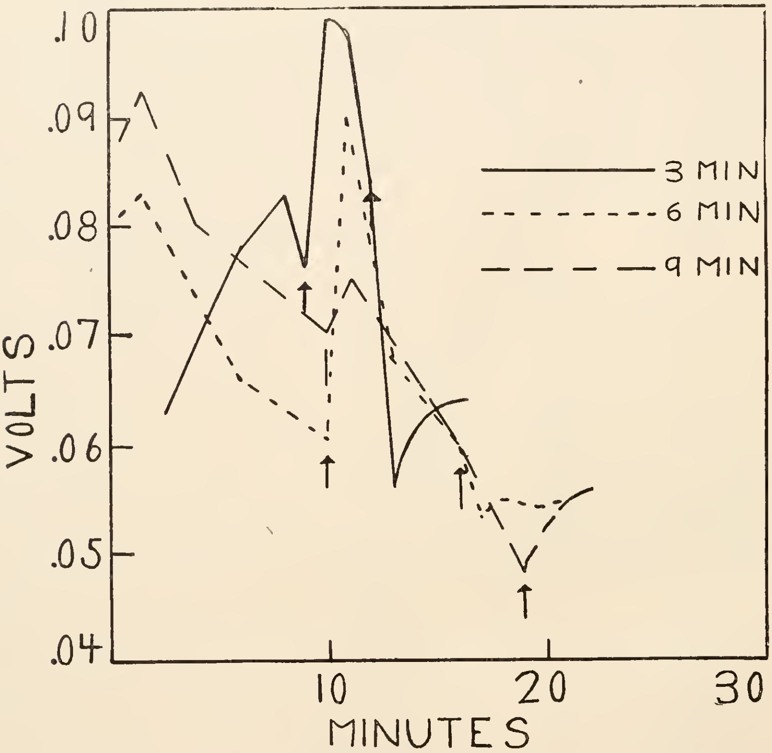

Fig. 11. Curve showing the effect of short exposures of cyanide to the P.D. of frog skin. The time between arrows represents the period of exposure to HCN, at other times the skin was exposed to borax buffer alone. Solid line represents a three minute exposure to cyanide; dotted line, 6. min. exposure and broken line 9 min. exposure. Abscissæ represents the exposure in minutes; ordinates, the P.D. in volts.

of exposure to KCN. It is well known that many anesthetics in dilute solutions act as protoplasmic stimulants and in concentrated solutions their toxic action is established (Osterhout, 9).

It is interesting in this connection to determine whether the rapid fall in the potential difference immediately following stimulation by dilute cyanide is due to a natural recovery to normal or to the toxic action of the cyanide. Cells were removed from the borax buffer solution at the end of a ten minute exposure and placed in a hydrogen cyanide solution of a concentration of $M/160$. A series of three experiments was conducted; in the first series the cells were removed from the cyanide solution at the end of three minutes and placed in a borax buffer, at which time the stimulation had reached its maximum and started to drop. In the second series the skin was allowed to remain in the cyanide solution for six minutes, then removed and placed in the pure buffer, at which time the drop following stimulation had reached the same reading as when the skin was first placed in the cyanide. In the third series the skin was exposed to the cyanide for a period of nine minutes. At the end of that time, the drop following stimulation had reached a point below the original P.D. The results of a typical series of experiments are plotted in Fig. 11. It is evident from this figure that after the removal of the skin from the cyanide solution, in the three and six minute exposure, the drop in P.D. continued until it had fallen below the P.D. obtained at the time the skin was placed in the cyanide solution; the P.D. then increased to normal. The nine minute exposure showed no further drop in the P.D. after being removed from the cyanide solution but an immediate recovery occurred. It is apparent from the data given that the drop in potential difference immediately following the stimulation was due not to the toxic action of the cyanide but to a natural return to the original reading and the toxic action did not take place until after the drop had surpassed the point where the skin was stimulated.

Effect of the pH of Cyanide Solution on the Potential Difference.

It is apparent from the data given in the first part of this paper that little or no HCN penetrates living membranes except in the form of undissociated molecules; thus it was deemed advisable to study the effect of dissociated and undissociated molecules of HCN on the potential difference of frog skin. Control experiments were conducted using borax-boric buffer free from cyanide at a pH of 6.8 and 8.5 to determine whether or not the hydrogen ion has any effect on the frog skin. Fig. 12 shows the results obtained. It is obvious that the two curves coincide very closely and that the hydrogen ion had no effect on the skin. It may be noted that the curves are strikingly similar to the control curves plotted in Fig. 9. The initial rise followed by a gradual decline was again manifested. Fig. 13 shows the results obtained by placing the frog skin "cells" in a solution of HCN in borax buffer at pH values of 6.8 and 8.5. The concentration of cyanide used was M/154. The temperature was constant at 22° C. ± 0.5°. The "cells" were placed for ten minutes in a borax buffer solution at pH values of 6.8 and 8.5 respectively; at the end of that time they were removed and put in a borax buffer containing

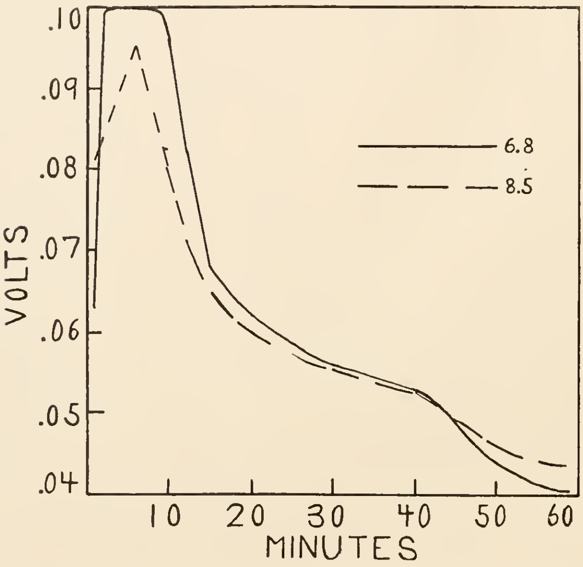

FIG. 12. Curve showing the effect of pH of the borax buffer on the potential difference of frog skin. pH valves 6.8 and 8.5. Abscissæ represents the exposure in minutes; ordinates, the P.D. in volts.

HCN at their respective pH values. The period of exposure to was greater at a pH of 8.5 than at 6.8. It is also apparent that the drop in the P.D. after a return to normal was somewhat more cyanide is represented on the curve by the time between the arrows. From Fig. 13, it is obvious that the initial stimulation pronounced under the acid conditions than under the alkaline.

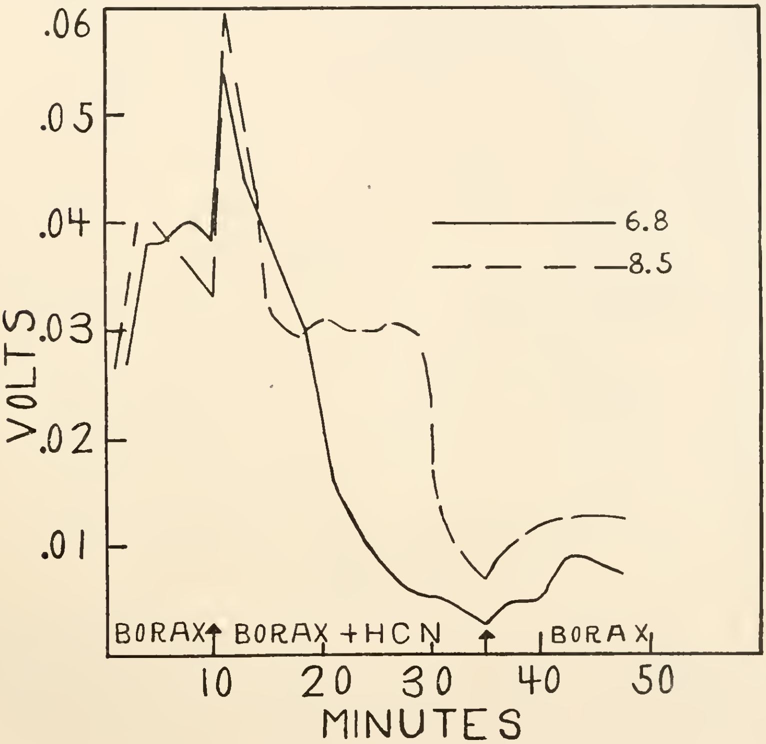

Fig. 13. Curve showing the effect of the pH of a solution of HCN in borax on the P.D. of frog skin. Period of exposure to cyanide represented as the time between arrows, at other times the skin was exposed to borax alone. pH values used were 6.8 and 8.5. Abscissæ represents the exposure in minutes and the ordinates the P.D. in volts.

There actually are fewer molecules of cyanide in a solution with a pH of 8.5 than with a pH of 6.8 (Fig. 1). So if the molecules were the toxic units, it would be expected that there would be less stimulation and more toxicity when the solution contains the greatest number of molecules than under the reverse conditions of less molecules and more ions. Such a condition was found to exist, as is evident from Fig. 13 where more stimulation and less toxicity occurred under the alkaline conditions than in the acid solutions.

In Fig. 14 are plotted the results of a series of tests using a solution of HCN in Ringer's solution. The pH of Ringer's solution was about 8.2; by the addition of HCl the pH was changed to 6.4. The same concentration of cyanide was used in each series (*M*/250). The "cells" were placed in Ringer's solution for ten minutes, then removed; one series was placed in a solution of HCN in Ringer's solution at a pH of 6.4 and the other series at a pH of 8.2. Potentiometer readings were taken every minute for 45 minutes. Again it is evident from Fig. 14, that there is greater stimulation and less toxicity under alkaline than acid conditions. In either case the initial stimulation was not as pronounced as in the experiments when borax buffer solution was used. It is, how-

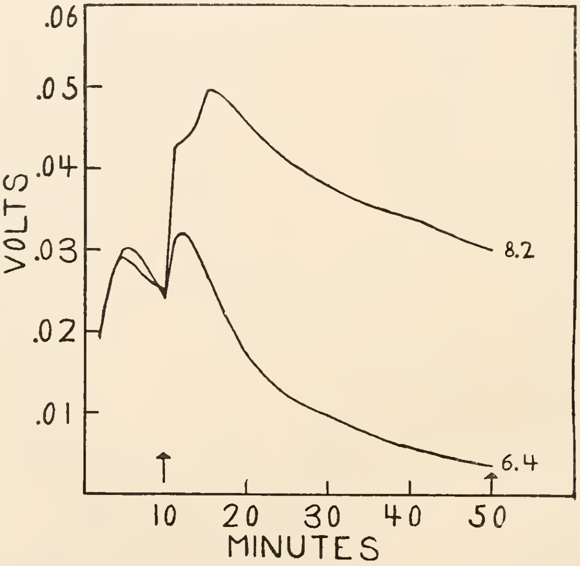

FIG. 14. Curve showing the effect of the pH of a solution of HCN in Ringer's solution. Exposure to cyanide represented by the time between arrows. First ten minutes skin exposed to Ringer's solution alone. pH values used were 6.4 and 8.2. Abscissæ represents the exposure in minutes and the ordinates the P.D. in volts.

ever, sufficiently marked to be significant. Figs. 15 and 16 show the results obtained when the pH was alternated from 6.6 to 8.2 and vice versa. The same piece of skin was used under the same conditions of temperature and concentration of cyanide; the concentration of cyanide was *M*/173, made by adding liquid HCN to a borax buffer. The skin was placed in a borax solution for ten minutes, then removed and placed in the cyanide solution at a pH of 8.5 for 15 minutes (Fig. 15). The characteristic curve was obtained—an increase in P.D. followed by a gradual decrease.

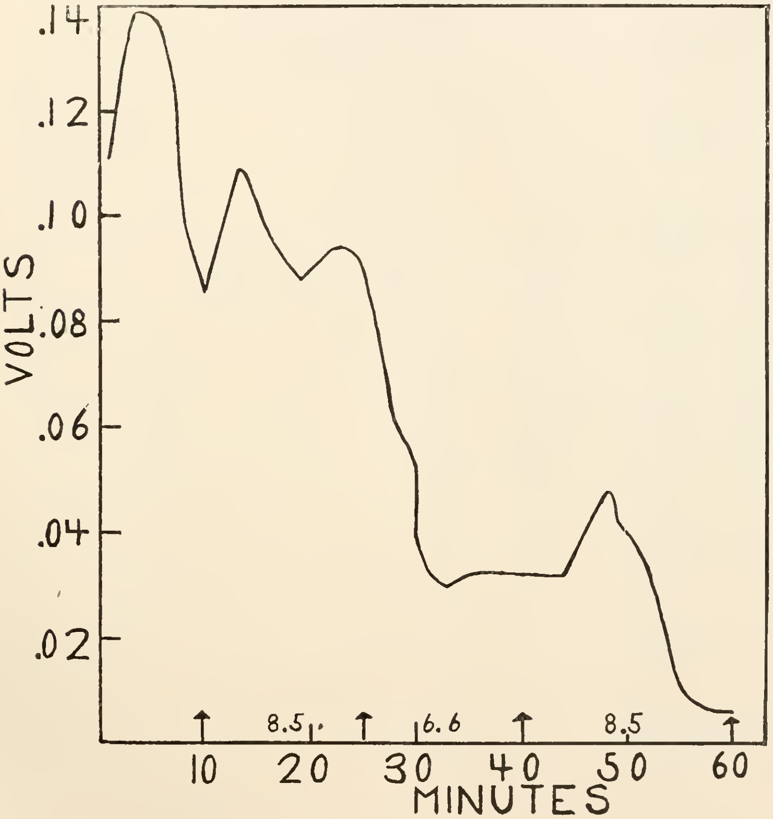

FIG. 15. Curve showing the effect of alternating the pH of a solution of HCN in borax on the same piece of frog skin. pH changed from 8.5 to 6.6 then back to 8.5. Period of exposure at various pH value indicated in curve. For the first ten minutes the skin was exposed in minutes and the ordinates, the P.D. in volts.

When the period of 15 minutes had elapsed, the skin was removed and placed in a HCN solution of the same concentration and at a pH of 6.6. It will be noted from Fig. 15 that a very sudden drop occurred in the P.D. as soon as the skin was placed in the solution at a pH of 6.6. At the end of 15 minutes the skin was again placed in the original HCN solution at a pH of 8.2.

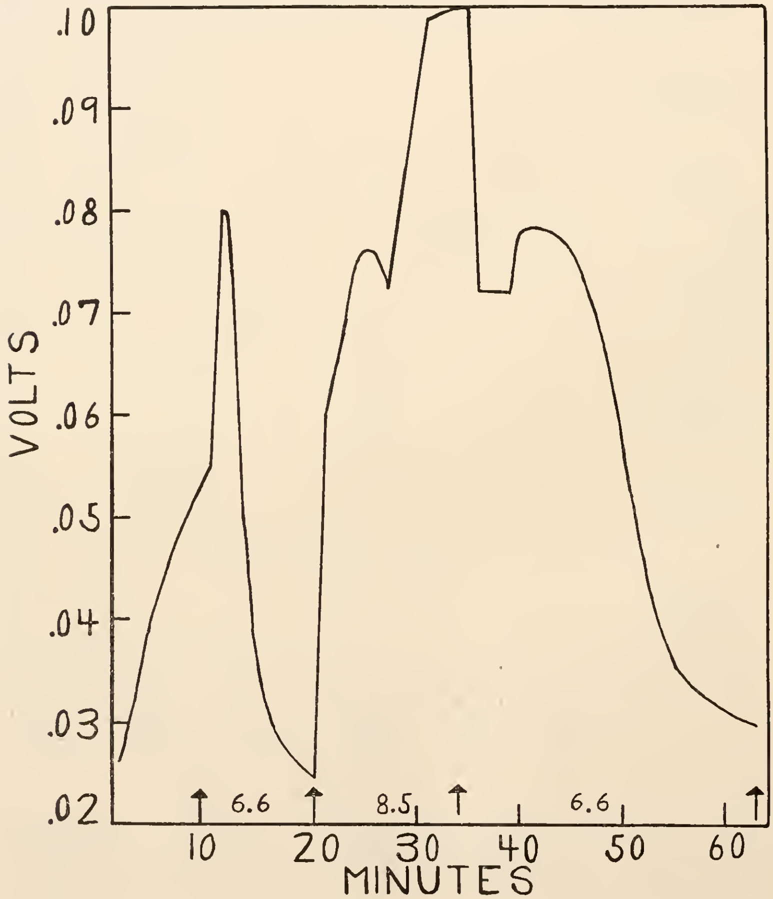

Fig. 16. Curve showing the effect of alternating the pH of a solution of HCN in borax on the same piece of frog skin. PH changed from 6.6 to 8.5 then back to 6.6. Period of exposure at various pH values indicated in curve. For the first ten minutes the skin was exposed to borax alone. Abscissæ represents time of exposure in minutes and the ordinates the P.D. in volts.

The P.D. gradually increased—suggesting a recovery—followed by a gradual decrease to the base line. The curve suggests, that at a pH of 6.6 the cyanide was more toxic than at a pH of 8.2 as was evident from the fact that the P.D. suddenly dropped when the skin was taken from a solution of a pH of 8.2 and placed in a pH of 6.6. When the skin was taken from a pH of 6.6 and placed in a solution of 8.5 a recovery occurred.

The conditions plotted in Fig. 16 are the reverse from those in Fig. 15. The skin after being placed in a borax buffer solution for ten minutes was removed and put in a cyanide solution of a pH of 6.6. The initial rise followed by a rapid drop was again obtained. When, however, the skin was removed from the pH of 6.6 and placed in a cyanide solution having a pH of 8.5, the P.D. rapidly increased until a point was reached above the initial stimulation suggesting a recovery and a stimulation. At the end of 15 minutes the skin was again placed in the original HCN solution at a pH of 6.6; a rapid fall occurred, suggesting a return to normal, followed by a rapid decline. As previously stated, 98 per cent. of the total cyanide is dissociated at a pH of 8.5 and thus the cyanide is present in the ionic condition. When the pH is 6.6 the cyanide is about 40 per cent. dissociated, therefore, the number of molecules and ions is about equal. From the data given, it appears that the cyanide is more toxic to the frog skin when the solution is acid than when alkaline. Thus, it seems that the molecule is actually more toxic than the ion. In dilute concentrations the physiological effect of hydrogen cyanide on frog skin is first a stimulation followed immediately by a rapid return to the original reading terminating in a toxic effect which will eventually prove fatal.

SUMMARY.

The experiments indicate that little or no HCN penetrates frog skin "cells" except in the form of undissociated molecules. The total amount of intracellular cyanide is proportional to the concentration of undissociated molecules in the external solution. The internal pH value of the "cell" has no effect on the penetrations of HCN through frog skin.

From a study of the effect of hydrogen cyanide on the potential difference of frog skin, it appears that dilute solutions of cyanide cause an initial stimulation followed by toxicity. As the concentration is increased, the stimulation is decreased and the toxicity is increased until a certain concentration of cyanide is reached where there is no stimulation but a marked toxic effect is evident.

The data also indicate that the molecule is more toxic than the ion.

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SEX RATIO IN *GAMBUSIA*.[1]

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Introduction.

The unequal division of the sexes in adult *Gambusia* and other viviparous minnows is a subject discussed by many writers. The present author has had an opportunity, in connection with the study of the value of *Gambusia* as an agent for the control of mosquitoes, to make many observations and to sex numerous fish consisting of both adults and juveniles and the results from this work form the basis for the present paper.[2] The material upon which the present study was based was obtained chiefly in three localities, namely, Augusta, Ga., Beaufort, N. C., and Greenwood, Miss. Therefore, the data, according to the most recent classification (Geiser, 1923, and Hubbs, 1926), are based on two species of *Gambusia,* namely *holbrooki* from Augusta and Beaufort, and *affinis,* according to Geiser, or *patruelis* according to Hubbs, from Greenwood. The present writer is unable to discuss the merits of the further division between *affinis* and *patreulis,* recently recognized by Hubbs. However, the differences in the structure of the distal part of the anal fin of adult males from Augusta and Beaufort (Atlantic drainage) and those from Greenwood (Misssissippi River drainage) are unmistakable and they are correctly described and figured by Geiser (1923). Furthermore, the present writer finds that when the rays in the dorsal fin are accurately and uniformly enumerated in both species, that is, when the last two rays that are more or less united, are counted either as one, or as two rays, a constant difference of one ray becomes evident.

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[1] Published by permission of the U. S. Commissioner of Fisheries.

[2] The writer wishes to acknowledge the very valuable assistance rendered by Irving L. Towers, recently junior aquatic biologist, U. S. Bureau of Fisheries, who was associated with the author for several years in the *Gambusia*-mosquito studies and who personally sexed many adult fish and made numerous observations herein recorded.

When counting the last, partly united, rays as two in 81 specimens examined from the Atlantic drainage (Augusta, Ga., Beaufort, N. C., Orangeburg, S. C., and Key West, Fla.) the dorsal fin constantly had 8 rays. Similarly, in 78 specimens examined from the Mississippi River drainage (Greenwood, Miss., Memphis, Tenn., Mound, La., and Little Rock, Ark.) 7 dorsal rays invariably were present. The variation of two and three rays reported in published works, therefore, probably is due chiefly to a difference in enumeration. The difference in the number of dorsal rays, then, is helpful in separating the otherwise bothersome females, as well as all juveniles which have not yet developed the external sexual characters.[1]

Although the data, forming the basis for the present paper, are founded upon two species of *Gambusia,* they will not be delt with separately, as no differences between the species with respect to sex ratio, neither seasonal, for adults, nor for young fish were noticed. The specimens, collected from 1921 to 1926, were taken in a large variety of waters, that is, ponds of various sizes (with and without vegetation) borrow pits, swamps (both fresh and brackish), ditches and sluggish creeks. The specimens, in fact, were taken in nearly every environment in which *Gambusia* lives. The data, therefore, are representative of animals living under a large variety of conditions, and not of any particular environment.

Although many authors, as already stated, have discussed the sex ratio in adult Pœciliids, little work has been done on the sex ratio of immature fish. The only account of the sex ratio of young Pœciliids, of any importance, that has come to the writer's notice is the one by Geiser (1924). Even Doctor Geiser's data are rather limited, yet they show quite accurately the sex ratio of young *Gambusia,* as will be demonstrated by the extensive data that are offered in a subsequent section of this paper.

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[1] It appears to be of interest to note here that a small collection (26 specimens) from Camilla, Ga., is at hand in which occur five females with only 7 rays in the dorsal fin, whereas all the others, including two males, have 8 rays. Geiser (1923) studied specimens from the same collection and, basing his identification on the minute structure of the intromittent organ of adult males, found them to represent the eastern form, *holbrooki,* although Camilla is in the Gulf drainage. It would now appear as if both species occur in this locality. Unfortunately no males with 7 dorsal rays are at hand with which to verify this identification.

Sex Ratio in Adult *Gambusia*.

The literature dealing with the sex ratio of adult Pœciliids, as previously indicated, is extensive and scattered and it will not be reviewed here. The reader, however, is referred to Dr. Samuel W. Geiser's admirable paper, " Sex-ratio and Spermatogenesis in the Top Minnow, *Gambusia holbrooki* Grd.," (1924) for a review of this literature and a concise discussion on the subject. It is sufficient to say here that the inequality of the sexes in adult fish is an unmistakable and a positive fact. As further proof, Table I., based on 103,150 fish, examined for sex, is offered. In explanation, it may be stated that the fish were collected either with a bobbinet seine or a dipnet made of the same material. Therefore, it was impossible for the smaller males to escape through the meshes of the nets.

In general fish having a total length of about 21 mm. (using " eye measure ") and over were considered adult fish and are included in Table I. The modified anal fin, serving as a copulatory organ, of course, was used in separating the sexes. That is, when a fish possessed a modified anal fin it was classed as a male, otherwise as a female. Many males, as will be explained later, undoubtedly were classed as females, because of the late development of the copulatory organ in some individuals. Occasionally this organ is fully developed when the fish is 18 mm. long and generally if not fully formed it is at least sufficiently developed to admit of recognition when the fish is about 21 mm. long. However, many exceptions are found, as is shown in the section of this paper dealing with the sex ratio of immature fish. It is enough to say here, that the disparity of the sexes, that is, the minority of males, is not as great as shown in Table I. The number of males, classed as females, nevertheless, is entirely too small wholly to account for the large inequality in the sexes, and the fact that the males are greatly in the minority among adult fish unquestionably remains.

It was shown by Barney and Anson (1921) that a " seasonal male frequency " takes place. The data presented in Table I. bear out the information offered by these writers, namely that the males are comparatively much more numerous during the fall, winter and spring than they are in midsummer. The data at hand are for the following months: June, July, August, September, October and December. Males were most numerous, as shown by these data, in June, when the ratio was 1 male to 2.54 females among 4,902 fish examined. In July the ratio, based on the examination of 17,941 fish, was 1 male to 5 females. In August the males were the fewest, as a ratio of only 1 male to 11.3 females obtained, as shown by the examination of 21,446 fish. In September the males began to increase, for a ratio of 1 male to 8.35 females was obtained from a total of 13,473 fish. In October the males increased still further, for 1 male to 2.75 females was found among 43,288 fish examined. A single day's collection, from Beaufort, N. C., taken chiefly in brackish swamps, for December is at hand, which consists of 2,100 fish. The sex ratio in this collection consists of 1 male to 3.6 females. The number examined obviously is too small and the environment too limited to be representative of the usual sex ratio prevailing at this season of the year. The average ratio of males to females for all fish (103,120) examined is 1 to 4.4.

The comparatively large seasonal difference in sex ratio in *Gambusia*, is very interesting. Barney and Anson (1921) offer the following explanation (pp. 64 to 66): "Further inquiry into the causes of the varying seasonal frequency of males and of the species, shows that in the summer there is a decrease in depth and area of all water systems studied. . . . This lowering of the water in the bayous and ponds eliminates all the shallow margins which *Gambusia* frequent and where they are largely immune from their enemies, and forces them into deeper water where the incline of the banks is steeper. This accordingly means much less natural protection than the minnows formerly had, since predaceous fish, now at a period of heightened metabolism and consequent rapid growth, are especially destructive and have a marked effect in lowering *Gambusia* frequency. Doubtless the *Gambusia* eaten by their predators at this time are nearly all females of large size, since the adult females, gravid at this season, are much larger, much slower in movement, and are more noticeable because of their black abdominal spots than the small, quickly moving, uniformly colored male. With the height of *Gambusia* frequency in late August (1919) occurs properly the height of the young *Gambusia* frequency. The young frequency then diminishes quickly, apparently due to the scarcity of breeding males during the spring and summer, and the resulting lowered birth rate. The height of *Gambusia* frequency is immediately followed by a considerable drop in water stage (surface water index) and accompanied by an increase in the percentage of other species, many of which are predators. The *Gambusia* frequency decreases by loss of adult females, while the male frequency accordingly increases." In the light of the present studies, as well as the tables presented by Barney and Anson (1921) this explanation is entirely inadequate, and in the main contrary to what actually takes place. If, in the thinning out process, more females than males were eliminated, then, certainly, there should be an abundance of males in July and August. That is, at a time when few young of the current season have become sexually mature and when it is usually quite possible to distinguish between the young of the current season and those of the previous year. Barney and Anson's table, as well as Table I. (presented herewith), however, show that the reverse unmistakably is true. Furthermore, it may be remarked here that the error in sexing, by means of external characters, very probably is smaller during July and August than at any other time. That is, fish of the previous season have become sexually mature and comparatively few young of the current season have reached a sufficiently large size to be considered adults. Therefore, comparatively few males, at this time, are classed as females.

How may the seasonal variation in sex ratio of *Gambusia* then be explained? It is shown in a subsequent section of this paper that males and females are equally proportioned among young *Gambusia,* and it already has been stated that a very large decrease in the proportionate number of males present takes place shortly before (July and August) the main body of young fish of the current season become sexually mature. A greater thinning out of adult males than of adult females, therefore, must have taken place. A discussion of the evidently much more rapid decrease among the males than among the females, when few young fish are maturing to take their place, is reserved for a later section. It appears to be sufficient to state here that the proportionately greater abundance of males which begin to appear in September are the result of young fish that are becoming sexually mature, for among these fish the males are quite as numerous as the females. After the following May or June nearly all of the fish, born the previous year, have become sexually mature and the young of the current season are still nearly all immature fish. Then the very rapid decline in the male population, already mentioned, takes place.

Further study has convinced the writer that the main reproductive season of *Gambusia* is much more limited than stated by him (1917 and 1921). In catching and transporting large numbers of fish while engaged in the study of *Gambusia* in its relation to mosquito control, it became evident that, although some gravid females are seen in early spring and during the fall,[1] the main reproductive season lasts only about three months, namely June, July and August. A very sudden decrease in the number of gravid females was noticed at Augusta, Ga. (where special attention was given to this matter for several years in succession) during the early days of September and in that locality, at least, the reproductive season may be said to end by Labor Day. The end of the spawning period evidently is not greatly influenced by temperature, as has been the contention of various writers, for the month of September at Augusta generally is one of the warmest of the summer. Furthermore, the writer has for two winters (1925–26 and 1926–27) kept two lots of adult *Gambusia,* containing a fair percentage of males, in the terrapin nursery house at the U. S. Fisheries Biological Station, Beaufort, N. C., which is kept at summer heat. Yet reproduction was not induced during the winter months. It was noticed, however, that gravid females appeared somewhat earlier in the spring in the lots in the terrapin house than among fish that wintered out of doors. The influence of temperature on reproduction in *Gambusia,* therefore, does not appear to be very pronounced.

The scarcity of gravid females in "midsummer" was noticed by Barney and Anson (1921). These authors thought this to be due to the scarcity of males, stating that such a "condition would not normally occur in midsummer with a proper relative number of males present." These authors probably overlooked the fact that copulation is not necessary between broods in *Gambusia,* nor in certain other Pœciliids, as it has been shown by Zolotnisky (1901), Philippi (1908) and Hildebrand (1917) that several broods of young may be produced by females after they are separated from males. For example, Hildebrand (1917) segregated some female *Gambusia* early in the spring, providing them with individual aquaria. One female, at least, produced five broods of young that summer, without further copulation. If copulation is not necessary during the summer, as appears to be the case, then certainly the scarcity of males cannot account for the cessation of reproduction during midsummer. Furthermore, the writer has held both sexes of *Gambusia* together in aquaria for a number of years and he has not noticed that the reproductive period is lengthened thereby. It appears to be logical, therefore, to conclude that the great reduction in the proportionate number of gravid females at the end of August is not due to the scarcity of adult males. On the contrary, it is the opinion of the writer that the animals by that time have expended all of the energy on reproduction they can afford and breeding ends, regardless of weather conditions.

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[1] In the extreme southern parts of the United States, as at Key West, some gravid females are present at all seasons of the year.

TABLE I.

THE SEX IN ADULT *Gambusia.*

Month.	Males.	Females.	Ratio.
June	1,385	3,517	1 : 2.54
July	2,960	14,981	1 : 5.06
August	1,742	19,704	1 : 11.31
September	1,440	12,033	1 : 8.36
October	11,614	31,674	1 : 2.75
December	455	1,645	1 : 3.6
Total	19,596	83,554	1 : 4.4

SEX RATIO IN IMMATURE *Gambusia.*

It already has been pointed out that the sex ratio in young fish has received comparatively little attention. This is rather surprising in view of the many accounts that have been published dealing with the sex ratio of adult Pœciliids. Perhaps this interesting problem did not receive more attention for the want of proper material and because of the difficulty involved in determining the sexes in young fish. Sexing immature fish, however, was found easier than the writer had anticipated, and a brief description is offered of the technique. pursued.

Sexually mature fish at first were dissected with the view of learning definitely the exact position of the gonads and to study the general appearance of these organs in fish in which the sex was definitely known. It was found that with scissors the tail and a part of the back of the fish might be removed by a single clip and by making this cut through the base of the anal and sloping it forward at an angle of about 45 degrees the viscera, after removing the peritoneum, was exposed while remaining intact. The sexual organs, lying dorsally of the visceral mass, may then be examined in position or removed for examination. It will be noticed at once that the ovary has a black membranous covering, whereas, the testes in preserved specimens are pale in color. The testes, although lying very close together and described as " fused " by Geiser (1924), nevertheless, show distinctly a median depression and on this line they may be separated with a sharp needle into two nearly equal parts. The ovary, on the other hand, is definitely fused, it has no median line of depression and cannot be separated into equal parts without causing rough, unequal breaks. The more prominent projection into the abdominal cavity of the interhæmal spines in the male is another difference that is usable. These characters can be used in sexing immature fish from 15 mm. and upward in length, as they are evident under a binocular microscope.

In sexing fish that are less than 15 mm. long it is necessary to be more careful in removing the minute gonads and for differentiation they must be placed on a slide. As it is necessary to use transmitted light, the contents have to be spread. This may be accomplished by placing a cover glass over the glands and tapping it lightly. It is desirable at first to mount and study the gonads of somewhat larger fish, that is, of fish large enough to admit of sex determination by the method described in the preceding paragraph. This is desirable, because confusion may arise from the fact that the sperm cysts might be regarded as eggs. It will be noticed, however, under proper magnification that the cysts are more opaque and granular in appearance than the ova and they do not have a central "nucleus" which is quite characteristic of the eggs. It is possible to sex *Gambusia* at birth, that is when only about 8 mm. long by this method. Consequently, no tedious processes of embedding and sectioning of gonads need be involved. After a little practice one becomes quite proficient in removing the organs, as well as certain of the diagnosis.

It of course is understood that the specimens, especially the very small ones, must be well preserved or sexing by the process described becomes difficult and unreliable. The best results were obtained by the use of 80 per cent. alcohol to which 40 per cent. formaldehyde was added in the proportion of about 30 cc. to 400 cc. of the alcohol. The strength of the preservative of course may be considerably varied according to the temperature. During cool weather 80 per cent. alcohol alone preserves specimens quite as well as the mixture of the two, in the proportions given, during hot weather.

The rapidity with which immature fish could be sexed, in the manner described, made it possible to examine a comparatively large number of fish, as shown in Table II. Few males have become sexually mature at a length of 20 mm. and no appreciable change in the ratio of males to females has taken place in fish of this size. This is brought out in Table II., in which fish, 20 mm. and under in length, are divided into two groups, namely those of 15.5 to 20 mm. being placed in one group and those of 15 mm. and under in another. It will be seen that in each group the males and females are very nearly equally numerous. This seems also to show that no thinning out of males takes place before they have become sexually mature.

The data for the next group consisting of larger fish (20.5 to 25 mm.) listed in Table II. are not representative of the sex ratio in unselected fish of that size. It is understood that many fish 20.5 to 25 mm. in length are sexually fully mature, the males being recognizable by the modified anal fin and the females, if not actually gravid, nevertheless, frequently have a small elongate blackish spot or line in the position where the prominent black spot is situated when the abdominal walls are distended with eggs and young. Such fish are not included in this group, as it is based only on specimens that had developed no external sex characters by means of which they could be definitely recognized. These data show that no hard and fast line, with respect to size, can be drawn between adult and immature fish, as the external sex characters are developed at a much larger size in some individuals than in others. In this group of large immature fish, based on 1,660 specimens, the males, however, are considerably in the minority, as the ratio is 1 male to 1.48 female.

A few immature fish of even a larger size than the group discussed in the preceding paragraph were found. For example, among 285 fish, ranging from 25.5 to 30 mm. in length which could not be sexed definitely from external characters, the ratio was 1 male to 6.12 females. In this connection it may be noted, however, that not a single immature male exceeding a length of 28 mm. was found.

The variation in size and age at which the anal fin in the male becomes differentiated, that is, when it is developed into an intromittent organ or "gonopod" (as designated by Geiser (1924)) already has been pointed out by Hildebrand (1917) and Mast in Barney and Anson (1921). The variation in size at which this organ may develop appears to be even greater than these writers supposed. It is evident from these data also that a considerable number of males are included among the females when all fish of about 21 mm. and over in total length are considered "adult," as the present writer did in sexing fish by the use of external characters, or as Barney and Anson (1921) did when they classed all fish of 15 mm. and over in length, to the base of the caudal, as adults. A considerable number of males, therefore, were classed as females in Table I. However, as stated elsewhere, the error in sexing is not large enough to cause the great minority of males that is shown in Table I. The greatest error in sexing, that is, the largest number of males classed as females, undoubtedly occurred during the months when the males were increasing most rapidly in proportionate numbers, as for example in September and October, for it is then that the largest number of young are maturing and many come within the "doubtful" size group. (The principal error is among fish 21 to 23 mm. in length.) Similarly, the error in sexing must be smallest when the fewest young fish are maturing, as in July and August when the greatest disparity of the sexes occurs. The uncertainty of the sex determination from an external examination of some of the fish, classed as females in Table I., therefore, accounts for a part of the difference in sex ratio shown but probably only for a small part of it.

It is believed that ample evidence has been produced by the data in Table II. to show that a *one to one* sex ratio exists among young *Gambusia.* It would appear also from these data that this one to one ratio obtains at least until the fish become sexually mature. It is evident from Table I., however, that a "thinning out" process takes place among adult males which is very much in excess of the decrease among the adult females. A discussion of this interesting phenomenon is offered in the following section.

TABLE II.

THE SEX IN YOUNG *Gambusia.*

Size of Fish.	No. of Fish Sexed.		Remarks.
	Males.	Females.	
15 mm. and under. .	239	232	
15.5 to 20 mm.	878	853	
20 mm. and under. .	181	210	This lot was not kept separate more definitely as to size in the original records.
Total.	1,298	1,295	Very few fish of 20 mm. and under are sexually mature. These data, therefore, show the sex ratio among young *Gambusia.*
LARGER IMMATURE *Gambusia.*			
20.5 to 25 mm.	672	988	Based on fish that were not yet sexually mature, although fish in these size groups usually have developed external sex characters.
25.5 to 30 mm.	40	245	
Total.	712	1,233	

A DIFFERENTIAL DEATH RATE OF THE SEXES IN *Gambusia.*

This subject is discussed at some length by Geiser (1924) who says, "The males are much less resistent to harmful environmental factors than females, and hence have a lower survival value." This author then continues to say that he has found males killed much more readily in " high temperatures, high H-ion concentration, oxygen deficiency and concentrations of KCN." He also found a much higher death rate among males than females in *Gambusia* held in containers for shipment, as well as when confined in aquaria. Further along in the same discussion this author writes, " The males are smaller and hence are more liable to be devoured by small predaceous fish than the much larger female. Gravid female *Gambusia* in aquaria, also, attack and frequently kill the males." The present writer is in entire accord with Doctor Geiser's views and the data presented herein appear to lend support to his contentions.

The present writer in connection with his work dealing with fishes in relation to mosquito control has had occasion to make shipments of hundreds of thousand of *Gambusia*. It was noticed from time to time, that among the dead the males usually appeared to be proportionately much more numerous than the females. It, of course, was impracticable to sex fish that were being transferred from one place to another for breeding purposes, hence the percentage of deaths of each sex cannot be given for such shipments. The writer is certain, however, that at least usually the death rate among the males was higher than among the females.

In a large series of experiments performed for the purpose of determining the best method of catching and handling *Gambusia*, the adult fish were sexed and for these the results follow. The effects of high temperatures, also the effects of crowding the fish, as well as the results to be derived from the use of varying amounts of water in containers holding fish, were tested. Furthermore, an effort was made to determine, by the number of fish that survived after several days of confinement under identical conditions whether catching by seine or by dipnet was preferable.[1] The results with respect to the death rate for each set of experiments will not be given separately, as it was higher for males than for females for every condition tested. In some individual lots proportionately fewer deaths occurred among the males than among the females. Generally the reverse, however, was true, as already stated and as shown by the data that follow.

[1] The practical results derived from the experiments referred to were reported by Hildebrand (1925).

A total of 41,073 adult fish, consisting of 7,337 males and 33,736 females, were used in the experiments described in the preceding paragraph. The loss among the males was 29¾ per cent. and among the females it was 22⅓ per cent. This mortality, as a whole, was very high, that is, much higher than usually occurs in *Gambusia* that are transported from one place to another, because many of the fish, for the sake of experimentation, were held under very adverse conditions. For example, in working with the effects of high temperatures the containers were set in the sun for the purpose of determining at how high a temperature the fish could survive. The males and females, however, were subjected to the same tests and, therefore, the data should be nearly as representative of one sex as of the other. Two discrepancies, nevertheless, may be mentioned. First, in some lots all the males died before the experiments were concluded. In such cases the mortality of the females (for additional ones usually died) is greater than it would have been, if the experiments had been terminated when the last male had died. Second, it is probable that some immature males that exceeded a length of 20 mm. died and, if so, they were classed as females. Therefore, the percentage of deaths given for the females is subject to a small error, as it is somewhat higher than it should be for direct comparison with the percentage of deaths among the males. It may be concluded, then, that the differential death rate among the fish used in the experiments was greater than indicated by the data.

The data show that under artificial conditions the death rate almost constantly was higher among males than among females, and it seems probable, that the males are generally less resistant to harmful environmental factors and this probably is true in nature as Geiser (1924) has contended, as well as under artificial conditions.

The present writer has no other pertinent data to offer bearing upon the cause or causes for the greater decrease in males than females, occurring annually. Geiser (1924) argues that the smaller males are more liable to be devoured by small predaceous fish than the much larger females and he also points out that the female, herself, is an enemy of the male. Barney and Anson (1921), on the other hand, argue (see quotation, p. 393) that the larger and more conspicuous female is the one suffering most from predatory fish. The writer believes that the former view may be correct but that in addition to fish, other natural enemies, such as water snakes, birds and insects should be considered as they all take part in destroying *Gambusia*.

CONCLUSION.

It has been shown quite conclusively that in immature *Gambusia* the sexes are evenly represented, as the 2,593 fish (all 20 mm. and under in length) that were examined, consisted of 1,298 males and 1,295 females. It was shown also, that a large "seasonal" variation in the abundance of males among adult *Gambusia* takes place, the data presented showing that the fluctuation in sex ratio may vary from 1 male to 2.5 females in June to 1 male to 11.3 females in August. Some evidence was produced tending to show that this great "thinning out" of males may be due to their lack of resistance to adverse environmental conditions, and the possibility that they are more extensively preyed upon by natural enemies, also, was advanced. What the greater mortality of the males may be due to, however, very largely, remains for further investigation.

Although male *Gambusia* are constantly fewer in number than females, it seems certain, nevertheless, that enough males for breeding purposes always are present. It has been shown that copulation between broods is not necessary, as females separated from males early in the spring produced young throughout the breeding season. In the spring when apparently the majority of the females are fertilized for the duration of the breeding season, the males are proportionately much more numerous. The greatest scarcity of males occurs in midsummer (July and August) when the females born the previous season have nearly all matured and quite certainly have been fertilized. At that time few males are needed. It, at least, is unquestioned that when conditions are favorable, *Gambusia* are quite capable of perpetuating their kind. Certainly few fish multiply more rapidly or become more numerous. The thinning out of males, therefore, appears to be nature's process of eliminating "surplus" animals.

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BIOLOGICAL BULLETIN

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THE RELATION BETWEEN THE RESPONSES BY *AMŒBA* TO MECHANICAL SHOCK AND TO SUDDEN ILLUMINATION.[1]

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The reactions induced in *Amœba* by mechanical shock are remarkably like those brought about by sudden illumination (Folger, '25, '26). In both cases the response consists of a cessation of movement, the animal remaining inactive for a short time, then resuming locomotion. In both cases a short period intervenes between the application of the stimulus and the response, and this period, the reaction-time, varies directly with the magnitude of the stimulation, becoming shorter as the latter increases; while the time during which the amœba is inactive, the period of quiescence, likewise dependent on the magnitude of the stimulus, becomes longer with increase of the latter. Moreover, in both instances a certain amount of time must elapse after a stimulus has been applied before the amœba will respond to a second stimulus. Thus, an exposure to light of sufficient duration to bring about a response must be followed by an absence of light or at least by a lowered intensity for a certain amount of time before the amœba will again respond to an increase in illumination. This occurrence of a period during which the amœba is apparently reverting to the condition it was in before stimulation, which I have called the period of recovery,[2] and the fact that a recovery occurs after a mechanical shock as well as after sudden illumination, is of value and has been used, as we shall see presently, for a study of the relationship which exists between the reactions caused by mechanical shock and those brought about by light.

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

[2] The term recovery has been used here simply because it is descriptive; refractory period has been employed and in the case of light dark adaptation. Recovery should not be confused with the resumption of protoplasmic flow by the amœba. The animal may have resumed locomotion and be moving

In a study of the influence of light on *Amœba* (Folger, '25) the question occurred: since the animal must recover from the effects of a sudden illumination before it will again respond to sudden illumination, must it also recover from the effects of a mechanical shock before it will respond to sudden illumination? The answer was clearly an affirmative one. In many instances the mechanical shock entirely inhibited a response to a sudden increase in luminous intensity, when the latter was applied shortly afterward. In these instances, however, the shock was brought about by moving the coverslip with the tip of a lead pencil, a method which, while it did produce undoubted results, is extremely crude. As a better means for controlling mechanical shock has since been used, the experiment dealing with the effect of this stimulus on the response to sudden illumination has been repeated and the results are presented in the following pages.

Moreover, if a mechanical shock preceding sudden illumination influences the response to the latter, the question also arises, is the reverse true? Does sudden illumination affect the response to mechanical shock? The results of experiments designed to answer this question are also set forth in this paper.

Materials and Methods.

Specimens of *Amœba proteus* were used in the experiments, raised in small glass vessels containing a culture solution formed by adding raw hay to distilled water. Large and active individuals were selected for experimentation.

Two sets of apparati were employed, one to bring about sudden illumination, the other to cause a mechanical shock. The latter was obtained by allowing a copper wire, weighing about 300 mg., to drop through a glass tube, 68 cm. in length, which was supported by a stand and clamps in such a manner that the weight

rapidly and still not have "recovered" from the effects of stimulation as indicated by the fact that it fails to respond to a second exposure to illumination.

struck one end of the slide containing the amœba. Light was procured from a 1,000-watt, 112-volt, cylindrical Mazda stereopticon lamp, and flashed upon the amœba by means of the plane mirror of the microscope, set at an angle of 45 degrees. An intensity of about 16,000 meter candles was employed. To observe the organism when it was not illuminated by the strong light use was made of a Spencer miniature substage lamp.

The amœba to be experimented on was placed in a drop of water on a glass slide, within a ring of vaseline, and beneath a thin coverslip which was supported on one side by a small glass rod. The microscope was so arranged that when the amœba was in position under the lens, light could be flashed upon it or a mechanical shock applied at the will of the investigator, and the effect of one stimulus upon the other noted.

Experimental Results.

Table I. illustrates the effect of mechanical shock upon the response to light. The experiment from which the data in this table were derived consisted of a number of tests, in each of which the amœba was exposed to strong illumination, and in every other one of which it was subjected to a mechanical shock before being illuminated. This shock was of sufficient magnitude to cause a cessation of movement, and the animal was exposed to light immediately on the resumption of flow. An interval of three minutes between tests permitted a recovery from the effects of previous stimulation. As shown in the table the amœba failed to respond to light 4 times out of 6 trials when a mechanical shock

Table I.

Illustrating the Effect of Mechanical Shock upon the Response to Sudden Illumination.

In each trial the amœba was exposed to strong illumination, and in every other one it was subjected to a mechanical shock before being illuminated. Three minutes were allowed between tests.

	Number of Trials.	No Reactions.
Sudden illumination alone	5	0
Sudden illumination following shortly after a mechanical shock	6	4

preceded the exposure to illumination, while it responded 5 times out of 5 trials when previous stimulation by mechanical shock was lacking. These results are entirely in accord with previous observations and leave no doubt that mechanical shock does affect the response to light.

Table II. is the record of an experiment in which the procedure in the experiment just described was reversed. Here tests in which the animal was stimulated by illumination and immediately on the resumption of movement given a mechanical shock alternated with tests in which a mechanical shock alone was used as the stimulating agent. As shown in the table, individual No. 1 reacted 5 times out of 5 trials when stimulated by mechanical shock alone, while it reacted to the same stimulus only 3 times out of 6 trials when the mechanical shock was preceded by ex-

Table II.

Showing the Effect of Sudden Illumination upon the Response to Mechanical Shock.

In each trial the animal was subjected to a mechanical shock, and in every other one it was illuminated before being exposed to shock. Three minutes were allowed between tests.

Individual No.		Number of Trials.	No Reactions.
1	Mechanical shock alone	5	0
	Mechanical shock following immediately after sudden illumination	6	3
2	Mechanical shock alone	6	1
	Mechanical shock following immediately after sudden illumination	8	4
3	Mechanical shock alone	6	1
	Mechanical shock following immediately after sudden illumination	6	5
4	Mechanical shock alone	4	0
	Mechanical shock following immediately after sudden illumination	6	4
Totals	Mechanical shock alone	21	2
	Mechanical shock following immediately after sudden illumination	26	16

posure to light. Individual No. 2 reacted 5 times out of 6 trials when subjected to mechanical shock alone, and only 4 times out of 8 trials when the mechanical shock was preceded by illumination. Altogether, the 4 animals used in the experiment responded to mechanical shock 19 times out of 21 trials when this stimulus did not follow an exposure to light, while they failed to respond to it 16 times out of 26 trials when it did follow illumination, thus indicating that exposure to light does influence the response to mechanical shock. Even more convincing, however, are the results of an experiment to be described in the next paragraph.

It has already been shown (Folger, '26) that the length of time that an amœba remains inactive after stimulation by a mechanical shock is markedly affected by the length of time that has elapsed since a previous mechanical shock, and that, within limits, this quiescent period increases with increase in the length of time since the previous stimulation. Thus, the period of quiescence resulting from a mechanical shock which follows 30 seconds after a preceding similar shock is not likely to be nearly so long as that brought about by a shock following after an interval of 60 seconds. Table III. records an experiment in which somewhat similar results were obtained, but in which the organism was first stimulated by light and then by mechanical shock. From the table it is seen that the amœba reacted only twice out of 4 trials when 15 seconds were allowed for recovery between stimulation by light and by

Table III.

Showing the Effect of Light on Mechanical Shock.

In each test the amœba was first exposed to light and then allowed to recover from the effects of this stimulus for the time indicated in the table, after which it was subjected to a mechanical shock. The period of quiescence, recorded in the last column, consists of the time during which the amœba was inactive after it had been subjected to a mechanical shock.

Time Allowed for Recovery Between Sudden Illumination and Mechanical Shock (Sec.).	Reactions.	No Reactions.	Average Period of Quiescence (Sec.).
15	2	2	4.5
20	3	0	5.8
25	4	0	9.1
35	2	0	20.0

mechanical shock, and that the period of quiescence amounted to 4.5 seconds. Twenty seconds for recovery resulted in 3 reactions out of 3 trials, with an average period of quiescence of 5.8 seconds, 25 seconds for recovery resulted in 4 reactions out of 4 trials, with an average period of quiescence of 9.1 seconds, and 35 seconds for recovery resulted in 2 reactions out of 2 trials, with an average period of quiescence of 20 seconds.

Thus it appears that not only does sudden illumination affect the response of an amœba to mechanical shock, but that it has precisely the same effect as another mechanical shock.

Discussion.

A similarity of response to various kinds of stimuli has been noted in other organisms. Ewart ('03) has collected considerable data concerning protoplasmic streaming in plant cells, especially in the cells of *Chara* and *Nitella*. He and others have found that the cells react in a very characteristic way to various stimulating agents, the response consisting, just as in *Amœba,* of a temporary cessation of movement. The best quantitative results were obtained by means of a mechanical shock, brought about by dropping a weight on the coverslip beneath which the streaming cells had been placed. By using weights of various sizes a gradation in the magnitude of the shock was possible. Ewart discovered that streaming did not stop immediately on application of the stimulus, but after the intervention of a reaction-time, which was longer after a light shock than after one of greater magnitude and which in the event of a sub-minimal stimulus might amount to 7 or 8 seconds. He found, furthermore, that the time during which movement remained suspended likewise depended on the magnitude of the shock, streaming recommencing much sooner after a slight shock than after a heavy one. Various other stimulating agents, including light, heat, electricity, and change in concentration of the surrounding medium, gave very similar results, although in no instance was so accurate a quantitative measurement obtained as with mechanical shock. Ewart also noted that the application of one kind of stimulus may tend to inhibit the response to another kind.

The refractory period used as a basis for the experiments presented in this paper, during which recovery from the effects of previous stimulation occurs, has long been known, especially when light has been used as the stimulating agent. In this case the term dark adaptation has been used. An animal must be dark-adapted before it will respond to illumination. This necessity for dark-adaptation has led to an explanation of the response to light which involves the presence in the organism of a substance capable of a reversible photochemical reaction (Mast, '07; Hecht, '18). The reaction is thought of as being initiated by the conversion of a photosensitive substance into its precursors, and dark-adaptation as the reforming of this substance from the precursors.

As we have just seen, however, recovery from the effects of a mechanical shock may be necessary before a response to sudden illumination is possible, and it would therefore seem that the same processes are involved in the refractory periods which follow the two types of stimuli. If so, since a photochemical reaction cannot occur in the case of mechanical shock, it is evident that in *Amœba,* at least, dark-adaptation must consist of something besides the reforming of a photosensitive substance from its precursors.

SUMMARY.

1. *Amœba* responds both to mechanical shock and to sudden illumination by a cessation of movement, which does not take place immediately on stimulation, but after a considerable reaction-time.

2. In both cases the reaction-time depends upon the magnitude of the stimulus, becoming longer as the intensity of the stimulating agent increases.

3. In both instances the time during which the amœba is inactive also depends upon the magnitude of the stimulus, becoming longer as the latter increases.

4. After an amœba has been exposed to light it is necessary that a certain interval of time elapse before it will again respond to sudden illumination. Likewise, after a mechanical shock the amœba must be allowed time for recovery before it will respond to a second shock.

5. After a response to light time must be allowed for recovery before the amœba will react to mechanical shock and vice versa.

6. The effect of one kind of stimulus upon a response to another kind leads one to infer that the processes occurring during the refractory periods following the reactions caused by mechanical shock and by sudden illumination are basically the same.

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CHROMOSOME NUMBERS IN THE GENUS *BURSA*.

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In a recent paper, Shull divides the genus *Bursa* into two groups on the basis of genetical studies. The *Bursa-pastoris* group includes six species and two subspecies, as follows: *Bursa bursa-pastoris* (L.) Britton, *B. bursa-pastoris apetala* (Opiz), *B. Heegeri* (Solms-Laubach), *B. occidentalis* Shull ined., *B. occidentalis madeiræ* Shull ined., *B. orientalis* Shull ined., *B. djurdjuræ* Shull ined., and *B. penarthæ* Shull ined. The *Rubella* group includes four species, as follows: *B. grandiflora* (Bois.), *B. rubella* (Reut.), *B. Viguieri* (Blaringhem), and *B. tuscaloosæ* Shull ined.

Crosses between members of the same group yield more or less fertile F_1 hybrids. Crosses between any species of one group and any species of the other group yield sterile hybrids in the F_1. Duplication of factors for certain characters is found in the *Bursa-pastoris* group of species, but not in the *Rubella* group.

Rosenberg (1904) had reported the haploid number of chromosomes of *Bursa bursa-pastoris* to be 16, and the diploid number to be 32. These counts were confirmed by Laibach (1907). Marchal (1920) reported 16 haploid chromosomes for *Bursa Heegeri,* and 8 haploid chromosomes for *Bursa Viguieri.* Knowing that two members of the *Bursa-pastoris* group possessed 16 haploid chromosomes, and that one member of the *Rubella* group possessed 8 haploid chromosomes, Shull suspected that the cause for the inter-sterility of these two groups might lie in a difference in chromosome number between the two groups. Since rather unusual opportunities were offered for securing material from Professor Shull's pedigree cultures of *Bursa,* I undertook a cytological examination of the various species of the two groups.

The cells selected for study were the pollen mother cells, since at the maturation divisions they offer relatively large cells with the reduced number of chromosomes, and can always be recognized. The material was fixed in Carnoy's acetic-alcohol-chloroform, and stained with Heidenhain's iron hematoxylin. No counterstain was used. The maturation divisions apparently consume very little time, for metaphase stages were never found in material which was fixed later than five minutes after collection. Since the buds are very small, dissecting out the anthers in this limited time was impracticable, so all of the unopened buds of a raceme were fixed at once. The raceme was then sectioned, usually at 6 micra. The cells at the time of the first maturation division are about 10 micra in diameter. It was possible to find cells cut at right angles to the spindle, so that the metaphase chromosomes were disposed in a horizontal plane. Counts were made in all of the species of the *Rubella* group. Marchal's count of 8 haploid chromosomes for *Bursa Viguieri* was confirmed, and 8 haploid chromosomes were found in the other three species. In *B. grandiflora* the diploid count also was made, and found to be 16, but somatic counts were not made for the other species of this group. In the *Bursa-pastoris* group not all of the species were counted, as several were unavailable, and in one available species no count was made. Rosenberg's count of 16 haploid chromosomes, 32 diploid chromosomes for *B. bursa-pastoris* was confirmed, and 16 haploid chromosomes found for *B. occidentalis, B. orientalis,* and *B. bursa-pastoris apetala.* As far as counted, these two groups are as sharply divided on the basis of chromosome numbers as on the results of breeding experiments.

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[1] This investigation was carried out principally in the cytological laboratories of Princeton University.

We may tabulate the chromosome numbers in the genus *Bursa* as follows:

	Haploid.	Diploid.	Author.
B. bursa-pastoris (L.) Britton	16	32	Rosenberg (1904), Laibach (1907), Author (1927)
B. Heegeri (Solms-Laubach)	16		Marchal (1920)
B. occidentalis Shull ined.	16		Author (1927)
B. Orientalis Shull ined.	16		Author (1927)
B. bursa-pastoris apetala (Opiz)	16		Author (1927)
B. grandiflora (Bois.)	8	16	Author (1927)
B. rubella (Reut.)	8		Author (1927)
B. Viguieri (Blaringhem)	8		Marchal (1920), Author (1927)
B. tuscaloosæ Shull ined.	8		Author (1927)

It is planned to continue this work next year when more material is available, completing the counts for the species of the *Bursa-pastoris* group, and examining a certain number of the inter-group hybrids. I had at first planned to study the details of the maturation mitoses, but the small size of the chromosomes and the difficulty of securing satisfactory material in the right stages of division make it unlikely that this will be attempted.

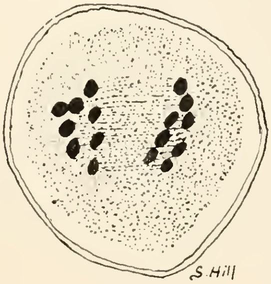

FIG. 1.

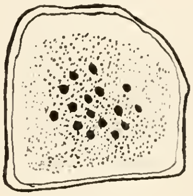

FIG. 2.

FIG. 1. Anaphase of first maturation division of *B. grandiflora*. × 3,000.
FIG. 2. Metaphase of first maturation division of *B. occidentalis*. × 3,000.

Figures are given for *B. grandiflora* and *B. occidentalis* only, but in the later paper figures will be given for all of the species counted.

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ULTRAVIOLET RADIATION AND THE FERTILIZATION REACTION IN *ARBACIA PUNCTULATA.*

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For a general consideration of the fertilization problem, the reader is referred to Chapter VIII., "Fertilization," by F. R. Lillie and E. E. Just, in Cowdry's "General Cytology," 1924; also to "Problems of Fertilization," by F. R. Lillie, 1919. In this paper, the studies of individual authors will be cited only where it seems desirable to refer to results which have a particular bearing on those obtained in the experiments about to be described.

The fertilization reaction may be modified by the direct action of a physical or chemical agent upon either or both of the sex components, or upon the zygote. Such interference results in the production of differentially modified larvæ, of irregular cleavage (often incomplete), of lack of membrane formation, or even of cytolysis. The magnitude of the injury is a function of the dosage and the time at which the exposure is made. This is easily demonstrable with *Arbacia* and ultraviolet radiation (Hinrichs, '26, *a, b*).

Furthermore, it is possible by means of radiation to interfere with the characteristic behavior of the sex cells, *e.g.,* the motility of the sperm is lost (Lillie and Baskervill, '22, and Hinrichs, '26*c*), and the agglutinability by normal egg-water is reduced or lost. The permeability of both sex components is altered, and the fertilizin-producing capacity of the egg is lost, following radiation.

Methods.—In the experiments involving the radiation of eggs, sperm, or egg-water, the material was placed in open dishes (immersed in a water bath) at a distance of 12.5 cm. from the lamp. (A few exposures were made at 23.0 cm. from the lamp, for comparison.) The radiation source was a Cooper-Hewitt quartz mercury-vapor lamp, operating at 110 volts D.C. and about 4 amperes.

Variations in technique will be noted as the various groups of experiments are described. The following topics are considered; radiation of unfertilized eggs, "egg-water," sperm suspensions, and agglutinated sperm; adsorption of the colloidal components of egg-water, and their partial recovery by HCl.

A. Experiments with Unfertilized Eggs.—Unfertilized eggs were radiated "dry," for varying periods of time (15 sec. to 20 min.) and tested for fertilizin production, as follows; equal volumes of sea-water (5–10 cc.) were added to equal quantities of eggs immediately after radiation and allowed to stand for from 15 min. to 2 hr. The water was then decanted off, and its agglutinating power measured. This was repeated from 1 to 5 times. The agglutinating power of a given sample of egg-water was measured by successively diluting the sample until it no longer gave a characteristic normal agglutinating reaction with a 1 per cent. sperm suspension. The last effective dilution is an

TABLE I.

ULTRAVIOLET RADIATION AND FERTILIZIN PRODUCTION.

Date.	Time after Exp. (Min.)	Length of Period of Radiation. (In min.)									
		0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	3	5	10	15	20
		A. U.									
8/26	30	6.0						15	30	15	
	60	1.5						—	1.5	—	
27	30	70				110	90	70	70		
	60	3				18		6	6		
28	15	60						30			
	30	7						7			
	45	—						—			
28	15	350				700			55		30
	30	60				60			6		1.5
	45	60				60			6		—
	60	60				30			6		—
30	15	600	600	600	1,200						
	30	"	"	"	"						
	45	"	"	"	"						
	60	"	"	"	"						
	240	1,200	2,400	2,400	4,800						

A. U.—Agglutinating units. Tests were made against a 1 per cent. sperm suspension.

expression of the agglutinating power in terms of "agglutinating units." This is the method used by Lillie in determining the agglutinating strength of a given sample of egg-water (F. R. Lillie and E. E. Just, '24, p. 487). A summary of a number of such experiments appears in Table I.

It will be seen that the rate of fertilizin production is at first increased, then decreased. In 3 experiments, the increase came with exposures of 1 minute or less. In the other two experiments, all exposures exceeded 5 min., and in one case the maximum increase came at 10 min. Successive tests for agglutinating power showed a rapid decrease in rate of fertilizin production, both in radiated and in non-radiated lots of eggs.

Samples of eggs fertilized with normal sperm at the time of testing for agglutinating power indicate a falling off in fertilizability and general viability running parallel to the decrease in rate of fertilizin production.

Table II.

Fertilizability of Radiated Eggs.

Min. Exp.	Min. after Exp.	A. U.	Stage of Development Reached. (In per cent.)			
			Morula.	Irregular Cleav.	Membranes Only.	Cytolysis.
0	15	350	98	1	1	—
	30	60	99	1	—	—
	45	60	97	2	1	—
	60	60	96	2	2	—
1	15	700	88	10	2	—
	30	60	50	45	5	—
	45	60	55	35	10	—
	60	30	35	53	12	—
10	15	55	68	5	3	24
	30	6	30	4	2	64
	45	6	25	10	5	60
	60	6	25	3	8	64
20	15	30	—	10	5	85
	30	1.5	—	3	5	92
	45	—	—	3	3	94
	60	—	3	5	3	89

A. U.—Agglutinating units.

The percentage of normal development is reduced by radiation (Hinrichs, '26*b*), and also by a delay in fertilization (F. R. Lillie, '15). In these experiments, cleavage is irregular and 50 per cent. of the eggs are cytolyzed following a 3 min. exposure, and from 50–75 per cent. are cytolyzed by exposures of 5 min. or longer. In general, where the percentage of abnormal cleavage and subsequent abnormal development is high, the fertilizin production is correspondingly low. Lillie and Just, '24, p. 487, call attention to the coincidence of the production of agglutinating substance by mature eggs with the fertilizable period of such eggs. (Lillie in 1914, and Just, in 1919, determined such a relation for *Arbacia* and *Echinarachnius,* respectively.)

The production of fertilizin is also decreased as membranes begin to form. A series of acid-treated eggs (with the jelly removed by the acid) showed no appreciable difference in the amount of fertilizin produced during successive intervals when compared with untreated eggs. (See discussion, Lillie and Just, '24, p. 492.) These tests were made to determine whether fertilizin was retained by the jelly and then given off into the sea-water in large amounts as though secreted by the jelly. The jelly appears to adsorb fertilizin, as will be described later, and the adsorbed fertilizin retains enough of its activity to attract and agglutinate sperm to the egg surface. Lillie suggests that the jelly acts as a protection against loss of fertilizin (Lillie, '19, p. 142). Sperm show a greater avidity for eggs radiated for short intervals than for non-radiated eggs, a fact suggesting an increased production of some substance which attracts them. (See also Table I.)

It will be seen that the rate of production of fertilizin in radiated eggs decreases as the time following radiation increases, and at a more rapid rate than in non-radiated eggs. The total production in radiated lots is also less than in normal eggs. This is probably due to an effect of ultraviolet radiation upon the egg cortex, which is supposed to be the seat of fertilizin production.

B. Radiation of Egg-water.—Egg-water contains several complex substances given off by the eggs. (See studies of Lillie and Just, '24, p. 483.) When such egg-water is exposed to ultraviolet radiation, its various components appear to be separately affected. The echinochrome pigment is faded, and the activating and agglutinating substances lose their effectiveness, all at different rates. Colorimeter measurements were made of the rate of fading of echinochrome. The results of such experiments are recorded in Table III.

TABLE III.

THE FADING OF ECHINOCHROME PIGMENT BY ULTRAVIOLET RADIATION.

Distance from Lamp.	Length of Period of Exposure. (In sec.)											
	0	2	4	5	6	8	10	15	20	25	30	35
12.5 cm........	*100%	100	94		72	62	50	52	49			
23.0 cm........	100%			81			67	51	49	46	35	35

* Results are given as per cent. of color remaining after each exposure. Non-radiated egg-water is used as the control.

Non-radiated egg-water was placed in one tube of the instrument and used as a color standard. Radiated samples were placed in the comparator tube. Three readings were taken for each sample, and the ratios of color intensities calculated. In each case, an average of the three results was compared with that of the non-radiated control. The final ratios appear in Table III. It will be seen that the ratios of color intensity in radiated lots as compared with color intensities in non-radiated lots decrease more rapidly as the length of the period of radiation increases.

Normal egg-water, when added to a normal sperm suspension, produces activation and agglutination of sperm, *i.e.,* the spermatozoa form regular spherical masses which persist for a short period of time, and then break up. The persistence of the masses is a function of the condition of the sperm, and of the concentration of active fertilizin in the egg-water. Radiation of egg-water causes a reduction and finally a loss of agglutinating power. Fewer dilutions of a given sample of egg-water are possible before the last effective dilution is reached. The results of radiating egg-water and the consequent loss of agglutinating power will be found in Table IV.

The rate of loss of agglutinating power does not exactly parallel that of the loss of color. It appears that ultraviolet radiation is more effective in accelerating the loss of agglutinating power than the loss of color. A 2 min. radiation at 12.5 cm. reduces the agglutinating power of a sample of egg-water by 50 per cent., but only a 6 per cent. reduction of color loss appears during the same period. In this case, 68 cc. of dry eggs were covered with enough sea-water in a graduated cylinder to reach the 250 cc. mark. The suspension was allowed to stand for 3 hr. before the egg-water was decanted off. This egg-water was then exposed to radiation for varying periods of time, and its agglutinating power tested against a 1 per cent. sperm suspension. In each case, the final agglutinating dilution was noted. (See Table IV.)

TABLE IV.

THE EFFECT OF RADIATION ON THE AGGLUTINATING POWER OF EGG-WATER.

Distance from Lamp.	Length of Period of Exposure. (In sec.)											
	0	2	4	5	6	8	10	15	20	25	30	35
	Agglutinating Units Remaining.											
12.5 cm. .	1,200	600	600		300	150	150		<1			
23.0 cm. .	1,500			900			850	300	150	88	63	18
	Agglutinating Units Lost. (In per cent.)											
12.5 cm. .		50	50		75	88	88		99+			
23.0 " .				40			44	80	90	94	96	99+
	Per Cent. Color Lost. (See Table III.)											
12.5 cm. .		0	6		28	38	50		51			
23.0 cm. .				9			33	49	51	54	65	65

Prolonged radiation of egg-water prevents the activation and agglutination of sperm, and may even produce a lethal effect on sperm. When radiated egg-water is added to normally agglutinated sperm, the clusters become " loose " and permanent, and the sperm inactive. Sperm inactivated by radiated egg-water may be reactivated by normal egg-water, so that normal but small, reversible clusters may form.

The agglutinating power of a given sample of egg-water may have been entirely lost following radiation, yet the same sample may still be capable of stimulating sperm to greater activity. Either the sperm-stimulating and sperm-agglutinating substances are not identical (Lillie and Just, '24, p. 483) or a lesser amount of the substance is necessary to activate than to agglutinate sperm. The former alternative is probably correct. (See also Glaser, '21, Woodward, '18, and Clowes and Bachman, '20.)

Temperature, in combination with the effects of radiation, is also a factor in determining the loss of agglutinating power and of color. To illustrate: samples of egg-water were radiated at 38°, 21°, and 4° C. Table V. records the results of such experiments. Only percentages of loss are given.

Table V.

Effect of Radiation at Various Temperatures.

Distance from Lamp.	Percentage Loss.							
	A. Agglutinating Units.				*B*. Color.			
	Min. rad.	Temperature			Min. rad.	Temperature		
		38° C.	21° C.	4° C.		38° C.	21° C.	4° C.
12.5 cm......	5	92	50	75	5	33	29	16
	10	100	90	88	10	64	52	59
	20	100	100	100	20	63	68	59
	Min. rad.	Temperature.						
		38.5° C	22.5° C	4° C				
23.0 cm......	5	50	50	88				
	10	99	90	99				
	20	99 +	99	100				

At any given temperature, the rate of loss of color is slower than that of agglutinating power; also, the rate of color loss is greater at higher than at lower temperatures, while the agglutinating power is lost more quickly at both high and low temperatures than at sea water temperature.

Normally the fertilizin content of egg-water is fairly stable, particularly if the egg-water is covered with a toluol film to minimize bacterial infection. (See also Lillie and Just, '24.) The greatest falling off in agglutinating power of such a sample comes during the first day or two, after which the agglutinating power remains fairly constant. (See Fig. 1.)

The radiation of egg-water produces no significant change in pH in either direction, although in some experiments a slight increase in acidity was noted. Following adsorption of the colloid components of egg-water by charcoal, an increase in alkalinity occurred.

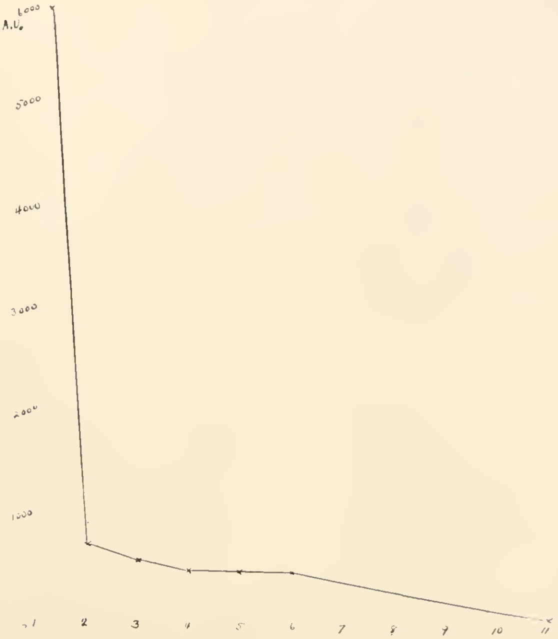

FIG. 1. Normal decline of agglutinating power of a sample of non-radiated egg-water. Ordinates, agglutinating units. Abscissæ, duration of experiment, in days.

Charcoal Adsorption.—Equal weights of animal charcoal, previously washed and dried, were added to equal quantities of a series of dilutions of egg-water whose agglutinating power was known. The charcoal egg-water mixture was shaken and then filtered through filter-paper, and its agglutinating power again tested against the same sperm suspension. The filtrate was colorless, and had lost from 90 to 100 per cent. of its agglutinating power, indicating a high adsorption coefficient. (See Table VI.) Both echinochrome and fertilizin had been adsorbed. (In this connection see also Glaser, '21*a*.)

It will be seen that relatively more fertilizin is adsorbed from samples of egg-water which have been diluted. The data for the experiment of 8/13 (Table VI.), are represented in Fig. 2. The agglutinating units for each dilution, before and after adsorption by charcoal, are indicated.

TABLE VI.

ADSORPTION OF FERTILIZIN BY CHARCOAL.
(Per cent. agglutinating units adsorbed.)

Date.	*A.U. —0.	A.U. —.1.	Conc. Sperm	Char-coal gm./cc.	Dilution of Egg-water.										
					0	.9	.8	.7	.6	.5	.4	.3	.2	.1	.05
8/12	1,125	5	4%	2 gm./100 cc.	95†	93	100	100	100	100	100	100	100	100	
13	384	—	1%	1 gm/100 cc.	93	96	96	95	95	98	98	99+	100	100	100
14	384	.8	1%	1.5 gm/100 cc.	43	86	84	91	89	88	84	95	99	99	
17	315	15	1%	200 mg./100 cc.	30	40	40	32	31	81	76	81	76	53	
18	192	1.5	1%	1 gm./25 cc.	54	44	68	64	58	88	84	89	92	89	
19	3,000	—	1%	1 gm./25 cc.	95	96	96	95	96	99	99+	99+	100	100	

* A. U.—0 number of agglutinating units at the start. (No dilution.)
A. U.—.1 number of agglutinating units at a dilution of 1 part egg-water to nine parts sea-water.
† Figures represent per cent. agglutinating units adsorbed.

Further evidence for the high adsorption constant of fertilizin is obtained as follows: when a suspension of fresh sperm is added to charcoal, the sperm swim actively about, often coming into contact with the bits of charcoal, but not remaining attached to them. If a few drops of egg-water are now added to this mixture, no agglutinated masses are formed in the spaces between the pieces of charcoal, but the fertilizin seems to be immediately adsorbed by the charcoal, and the sperm are attracted by it, and become agglutinated to each other in an irregular film around the bits of charcoal, and remain there. The masses are irreversible and permanent, due probably to the high concentration of still active fertilizin about the charcoal, and the lack of sufficient activating substance to stimulate the sperm to activity. The activating component of egg-water appears not to be adsorbed by charcoal, or less so than the agglutinating component.

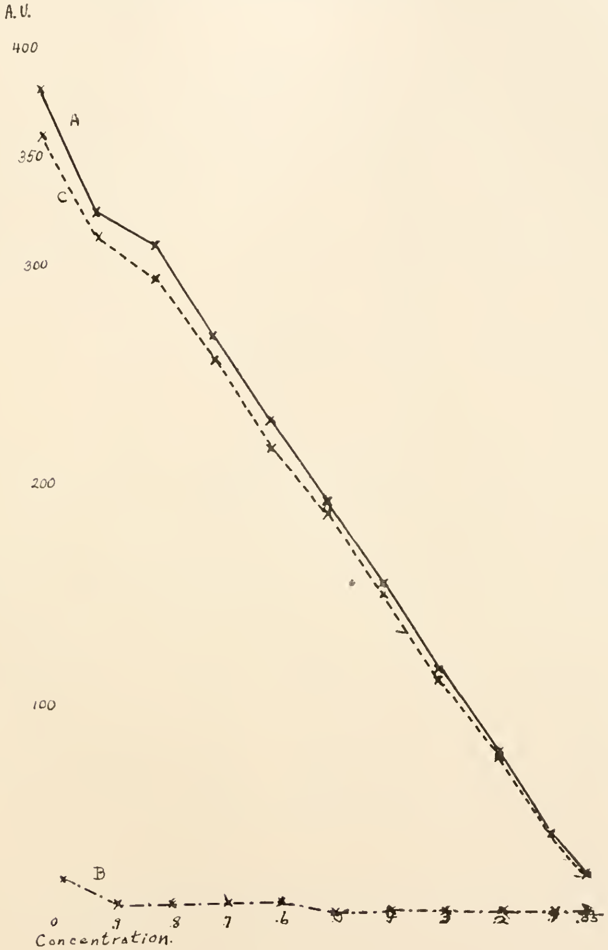

FIG. 2. Adsorption of fertilizin on charcoal. Ordinates, agglutinating units. Abscissæ, concentration of egg-water used. A. U., agglutinating units. *A*, agglutinating units in sample before adsorption. *B*, agglutinating units in sample after adsorption. *C*, agglutinating units adsorbed.

When a few drops of concentrated sperm are added to egg-water containing a small amount of charcoal, the sperm immediately attack the bits of charcoal and rotate them about much as they do ripe eggs. The retention of considerable activity by adsorbed fertilizin suggests its enzyme-like nature. Such behavior has been noted for pepsin by Kikawa, '23. Richards and Woodward, '15, and Lillie, '21, have ascribed an enzyme-like nature to fertilizin.

When ripe unfertilized eggs are added to a charcoal-fertilizin system, and then fresh sperm are added, the sperm show a preference for the eggs, rather than for the bits of charcoal. The concentration of active fertilizin is probably greater about the egg-surface due to continued production of fresh fertilizin by the egg.

It was possible to recover a small amount of the adsorbed fertilizin by washing the charcoal with slightly acidified sea water. (See Table VII.) Glaser, '21*b*, reports the recovery of some fertilizin by HCl. Neither iso-amyl alcohol nor saponin were effective in displacing the fertilizin from adsorption.

TABLE VII.

THE RECOVERY OF FERTILIZIN ADSORBED ON CHARCOAL.
($\frac{N}{10}$ HCl was used.)

Before Adsorption.		After Adsorption		% ads.	pH of Wash Water.	A.U. after Washing.	% Recovered.
pH.	A.U.	pH.	A.U.				
7.20	160–320	7.70	20–40	87.5	3.8	1 —32	11.4
7.45	3.500	8.0	150	96	6.8	—	—
8.00	1.250	8.1	300	76	6.7	3	1.
7.45	3.500	8.0	150	96	6.7	12	8.
7.65	1.500	7.8	1.5	99.9	7.1	—	—
7.80	150	7.8	—	100.	6.6	—	—
7.35	1.500	7.8	6	99.6	8.1	—	—
6.60	300	6.6	12	95.	8.2	—	—

When a charcoal-fertilizin system is washed with sea-water, the filtrate will be found to activate but not to agglutinate sperm. Apparently the activating substance is not adsorbed to the same degree as are the other substances in egg-water.

There seems to be an optimum pH range for the adsorption for fertilizin by charcoal, as well as for its effectiveness as a sperm-agglutinating agent. This optimum pH range, like that found by Clowes and Smith for fertilization, hovers around neutrality. (See Clowes and Smith, '22, '23, Smith and Clowes, '24, *a, b, c.*)

Measurements of pH in these experiments on fertilizin were made by the use of indicators. In the region of 6.8 to 8.2, from 75–87.5 per cent. of the fertilizin was adsorbed. In another series, at 7.5 to 7.9, the percentage of adsorption was from 96–100.[1] (See Table VIII.)

TABLE VIII.

EFFECT OF pH ON ADSORPTION.

Before Adsorption.		After Adsorption.		Per Cent. Agglutinating Units Adsorbed.
pH	A.U.	pH	A.U.	
9.8	120	9.8	120	—
9.0	120	9.0	60	50
8.2	120	8.2	30	75
7.5	120	7.8	15	87.5
7.45	240	7.8	30	87.5
7.2	240	7.7	30	87.5
7.1	240	7.5	30	87.5
7.0	120	7.45	15	87.5
6.8	60	7.2	15	75
5.4	30	6.0	15	50
4.8	30	6.0	30	—
3.6	6	4.4	—	—

Radiation of Sperm Suspensions.—Earlier work with *Arbacia* sperm has shown that a loss of motility and a reduction of fertilizing power follow the exposure of sperm suspensions to ultraviolet radiation. (Lillie and Baskervill, '22, Hinrichs, '26*c.*) In these earlier experiments, it was also noted that radiated suspensions of sperm had lost some of their opalescence and appeared cleared in transmitted light. This was probably due to the settling out of suspension of irregular aggregations of sperm; such clumping or agglutination results from exposure to ultraviolet radiation. (Hinrichs, '26*c.*) In the present series of experiments with sperm, it was noted that the decrease in opacity to light was proportional to the dosage of radiation a given sperm suspension had received. The results are recorded in Table IX.

The relative opacity was measured by means of a nephelometer. A 1 per cent. sperm suspension was used as stock, and from 1 to 20 drops thereof per 100 cc. of sea-water were exposed to radiation at 12.5 cm. from the lamp, for periods of from 15 sec. to 15 min. As in previously reported experiments, the more dilute suspensions were more susceptible to changes induced by radiation, and the effect was graded by exposure.

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[1] More work is planned on this phase of the problem.

Table IX.

Loss of Opalescence of Sperm Suspensions Following Radiation.

Time Exp. in Sec.	Concentration of Sperm. (Drops of 1 Per Cent. Suspension per 100 cc. Sea-water.)					
	20	15	10	5	3	1
15	*1.12	.81	.90	.97	1.19	.90
30	1.04	.84	.95	1.15	1.08	1.02
60	1.17	.87	.75	.97	1.33	1.00
180	1.11	.85	.94	1.05	.81	.83
300	1.01	.84	.77	.82	.65	.59
600	.85	.65	.68	.51	.61	.32
900	.69	.57	.46	.53	.41	.24

* Figures represent relative opalescence of radiated sperm compared with non-radiated sperm.

There are characteristic differences between normally agglutinated masses of sperm, and those induced by ultraviolet radiation. The latter are irregular and permanent, and the individual sperm inactive. The whole clump, in each case, appears to have been formed by sperm which had become agglutinated to each other, and had almost immediately lost their motility, remaining applied to each other by sticky surfaces. Such masses resemble the ones described by various other investigators and are generally known to be toxic and irreversible. (For discussion, see Lillie and Just, '24, p. 489.)

When masses of sperm, which had been normally agglutinated by egg-water, are radiated, several things may happen, according to the intensity and duration of the radiation, and the time of its application with respect to the "age" of the agglutinated mass. When freshly agglutinated sperm are but slightly radiated, the clump breaks up almost immediately. If a slightly longer exposure is given, the sperm appear to be paralyzed and the mass becomes permanent, and more or less irregular in shape.

When sperm are slightly radiated, and then treated with normal egg-water, the onset of agglutination is delayed. Motility is greatly decreased. Lillie, '12, and Loeb, '14, found the rapidity of the onset of agglutination to be a function of sperm activity. The time during which the agglutinated masses remain intact also decreases with increased dosage. (See Fig. 3.)

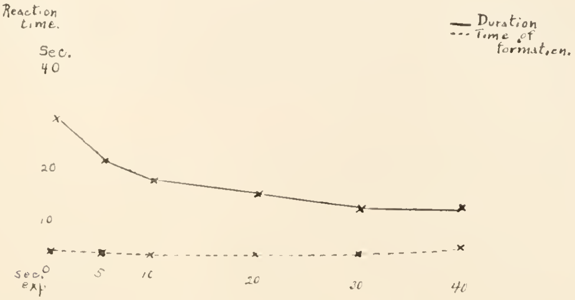

Fig. 3. Delay in agglutinating reaction following radiation of sperm. Ordinates, number of seconds elapsed before the onset of agglutination with normal egg-water. Abscissæ, length of period of exposure, in seconds.

The same conditions are found when egg-water is radiated for a short interval, and then used to agglutinate normal sperm. Its agglutinating capacity is reduced. Agglutination is delayed, and the masses persist for a shorter period of time than when normal egg-water is used with normal sperm. Longer exposures of either egg-water or sperm, previous to mixture with normal sperm or egg-water, respectively, produce larger irregular permanent masses which "run into each other" forming a kind of reticular mass throughout the suspension.

Table X.

Loss of Agglutinability of Sperm Suspensions Following Radiation.

A.		*B.*			
Time Exp. in Sec.	Greatest Effective Dilution.	Conc. Sperm in Per Cent.	Greatest Effective Dilution.		% Loss.
			Non-rad.	Rad.	
0	4,000–8,000	2.5	8,000–16,000	8,000–16,000	—
15	2,000–4,000	2.0	4,000–8,000	2,000–4,000	50
30	200–300	1.5	500–1,000	40–80	92
60	20–40	1.0	100–200	0–10*abn.	96.7
120	—				

A. Sperm in concentration of 3 drops of 1 per cent. suspension per 10 cc. sea-water.

B. Sperm in various concentrations radiated for 30 sec. each. * *abn*-aggregates were abnormal in appearance, and were permanent and "stringy."

Since the normal agglutinability of sperm suspensions is reduced by ultraviolet radiation, a stronger concentration of egg-water is necessary to produce agglutination in radiated sperm. Table X records the results of such an experiment. While the control sperm may be agglutinated with egg-water diluted about 6,000 times, sperm radiated for 60 sec. will agglutinate with egg-water diluted only about 30 times. Radiation for 120 sec. reduces agglutination to zero.

Radiated sperm suspensions show no change in pH detectable by the indicator method. Higher concentrations of sperm show a slight increase in relative viscosity, as measured by a stalagmometer. This bears out the suggestion made above regarding the increased adhesiveness of sperm heads in aggregations appearing in radiated suspensions. Apparently ultraviolet radiation changes the consistency and permeability of the sperm surface causing the sperm to become more " sticky." Sampson, '26, describes a similar change in the consistency of sperm agglutinated by normal egg-water. Glaser, '14*b*, found that egg extracts increased the permeability of cells. Other workers have reported similar results.

There appears to be a decrease in surface tension following the radiation of sperm suspensions. The relative permanence of foam structure formed the basis, for the above suggestion, following Bartsch, '26. 25 cc. of each of the various sperm suspensions were shaken in small bottles until frothy. Examinations were made after 30 and 90 minutes. It will be seen from Table XI. that the stronger suspensions of sperm had more froth than the more dilute suspensions, and that in both strong and dilute suspensions, the number of bubbles was increased by radiation, as indicated by the number of ×'s in the table.

General Discussion.—From the fact that the degree of viability and fertilizability of *Arbacia* eggs show a parallelism with the power to produce fertilizin, it may be argued that fertilizin plays an essential rôle in the fertilization reaction. (See Lillie and Just, '24, p. 486.) There appear to be two distinct substances yielded by the egg to the sea-water, one of which stimulates the sperm to greater activity, while the other agglutinates the sperm, perhaps by so affecting the sperm-head surface as to cause it to become more permeable to a viscous substance contained in the sperm, and given off into the sea water. Popa, '27, has recently described such a substance in *Arbacia* sperm.

Table XI.

Permanence of Foam Structure in Sperm Suspensions Following Radiation.

Length of Exposure.	*A.*			*B.*		
	Number of Drops of 1% Sperm Suspension per 100 cc. Sea-water.					
	30	15	5	30	15	5
0 min. . . .	×××	××	Scarce	××	Very few	Scarce
1 " . . .	×××	××	Scarce	××	Very few	Scarce
10 " . . .	××××	××××	××	×××	××	×
30 " . .	××××	××××	××	×××	××	×

A. Examined after 30 min.
B. Examined after 90 min.

Ultraviolet radiation likewise alters the permeability of the sperm surface, producing aggregates of sperm which, however, are not reversible, differing in this respect from the agglutinated masses produced by the action of normal egg-water upon normal sperm.

Certain of the above-described characteristics of fertilizin suggest an enzyme-like nature of the substance. Its high adsorption constant, the retention of some of its activity while in the adsorbed state, its displaceability by acids and not by fat-solvents, and an optimum pH range for activity and for adsorption have been described.

Tchachotine, '21, ascribed a sperm-agglutinating power to the gelatinous egg envelope. Although sperm do attach themselves in large numbers to the jelly layer of *Arbacia* eggs (if higher concentrations of sperm are used), this is probably due to the fact that the fertilizin is adsorbed by the jelly, and still retains some of its agglutinating power. A similar condition exists in the experiments described above, in which bits of charcoal with their adsorbed fertilizin behaved like artificial eggs, and attracted and held active sperm agglutinated in a film about them.

Glaser, '14*a*, found that sperm which had been paralyzed could be reactivated, but not reagglutinated. Sperm treated with egg-water which had been strongly radiated become inactive, but may be reactivated by normal egg-water and to some extent reagglutinated. The sperm exudate had apparently not been entirely "fixed" by the egg-water, even though the sperm had become inactive. This is further evidence of the presence of two substances in egg-water, one responsible for activation, and the other for agglutination of sperm. Two other bits of evidence cited above also bear out this suggestion. Radiation may remove the agglutinating power of a given sample of egg-water, yet it may still retain its power to activate sperm. Also, egg-water adsorbed on charcoal does not lose its activating power, for when the charcoal and egg-water mixture is washed with sea-water, and the filtrate added to fresh sperm, the latter are activated but not agglutinated. Apparently the activating component of egg-water is not adsorbed by charcoal, or less so than the agglutinating component.

The colloid components of egg-water are best adsorbed within an optimum pH range which hovers about the point of neutrality. Recovery by acid is possible, and increasing the acidity of the egg-water beyond the optimum range, lowers its adsorbability by charcoal. This may depend on a reversal of the charge carried by the fertilizin particles, but further experimentation on this point is necessary. Saponin does not displace the fertilizin from adsorption. Radiation of fertilizin causes a slight increase in acidity. This is in accord with the findings of Stedman and Mendel, '26, for protein solutions and distilled water.

The echinochrome pigment in egg-water is faded by ultraviolet radiation. Its function is a rather uncertain one. Glaser, '21*a*, suggested that this substance acts as a stabilizer for fertilizin. It has been found to have a certain amount of photodynamic activity. (See R. S. Lillie and Hinrichs, '23.) The pigment is also adsorbed by charcoal, as shown in the above experiments, and by Glaser in 1921. (Glaser, '21*a*.) It is probably colloidal in nature.

The production of fertilizin by mature eggs has been discussed above at greater length. F. R. Lillie associates fertilizin production with viability of eggs as follows, "When an egg ceases to produce the sperm-agglutinating substance, it has lost its capacity to be activated." (Lillie, '19, p. 240.) A reduction of fertilizin production following a temporary slight acceleration is probably due to the direct action of ultraviolet radiation upon the egg cortex. R. S. Lillie and Baskervill, '22, showed that radiation was effective in its direct action on the egg surface. Radiation induces membrane formation and fertilizin production is known to decrease as membranes begin to form.

Normal active sperm show an increased avidity for eggs which have been radiated a short time. Apparently this is due to the fact that ultraviolet radiation increases the permeability of the egg cortex, and liberates fertilizin to a greater degree than that found in normal eggs.

Normal sperm bear a substance necessary for fertilization which is given off into the medium, and after a certain period of loss of this substance, the fertilizing capacity of sperm decreases. Ultraviolet radiation augments the normal rate of loss of this substance from the sperm. (For a review of literature bearing on this subject, see Sampson, '26, and Hinrichs, '26*c*.) When radiation is long enough continued, sperm lose their capacity for normal fertilization as shown by the loss of ability of eggs, fertilized by them, to produce membranes, to cleave normally, and to develop into normal larvæ (Hinrichs, '26*b*). There is a corresponding loss of agglutinability of sperm by normal egg-water. Radiated sperm require a higher concentration of fertilizin to produce agglutination than do normal sperm. The same is true for sperm which have been standing for some time after removal from the gonad. (See Lillie and Just, '24.) Lillie and Just also report that the rapidity of onset of agglutination of sperm by means of normal sea-water is a function of the motility of the sperm. Radiation reduces sperm activity and also lengthens the time of onset of agglutination.

Radiation produces an increase in the viscosity of sperm suspensions. Mond, '22, and Wels, '23, report similar findings for the effects of ultraviolet and x-radiation upon proteins. In radiated sperm, this increase in viscosity, together with a reduction in motility which follows, is probably responsible for the permanence of clumps which are formed as the result of radiation alone. When normally agglutinated masses are radiated, the clumps become permanent, due to the increased adhesiveness and loss of motility of individual sperm. Also, radiated sperm produce reticular, stringy masses on the addition of normal egg-water. Here again, the increased stickiness of the sperm and the loss of motility are a result of radiation.

I wish to acknowledge my gratitude to Dr. R. S. Lillie for his coöperation in the study of this problem. The experiments were made at Woods Hole during the summer of 1926.

Conclusions.—I. Earlier work with ultraviolet radiation and *Arbacia* germ cells has shown the following results: (*a*) Radiated eggs fertilized by normal sperm, normal eggs fertilized by radiated sperm, or radiated zygotes produce differentially modified larvæ, (*b*) Radiation of normal sperm causes a reduction and loss of motility and fertilizing power, and (*c*) Radiation of normal sperm suspensions causes sperm to form irregular, permanent aggregates. Continued radiation kills the sperm.

II. The present experiments have added the following:

(*a*) The radiation of normal eggs produces at first a slight increase, then a decrease, and finally a complete loss of the power of producing fertilizin. There is a parallel loss of viability of the eggs as measured by their fertilizability and ability to develop normally.

(*b*) The radiation of normal egg-water produces a fading of echinochrome pigment, and a reduction of the agglutinating power of fertilizin. The two are affected at different rates. The additive effect of temperature and radiation is more rapid in its action upon fertilizin, than upon echinochrome.

(*c*) The agglutinating power of egg-water is lost before its sperm-stimulating power, suggesting that two distinct substances may be concerned.

(*d*) There is an optimum pH range, around the point of neutrality, for the agglutinating action of egg-water. A similar range is present for the adsorption of fertilizin by charcoal. Adsorbed fertilizin retains some of its agglutinating power. The enzyme-like character of fertilizin is indicated. Adsorbed fertilizin may be displaced from adsorption by a slight acidification, but not by the addition of surface-active compounds. Fertilizin has a high adsorption constant. For this reason, fertilizin is probably adsorbed by the egg surface to an extremely high degree, and usually not enough of it is present in an active state in the spaces between eggs to produce typical agglutinated masses of sperm, when eggs are inseminated in fingerbowls in the laboratory.

(*e*) Ultraviolet radiation produces a slight increase in the pH of egg-water.

(*f*) Sperm suspensions become more translucent as a result of radiation, in consequence of the formation of permanent irregular aggregations of sperm which settle out of suspension.

(*g*) The more concentrated suspensions of sperm show an increase in viscosity and a decrease in surface tension following radiation. The increase in viscosity is associated with the formation of aggregates of sperm.

(*h*) Radiated sperm undergo a reduction of agglutinability by normal egg-water. The onset of agglutination is delayed, and the duration of the phenomenon is shortened.

(*i*) The more dilute suspensions are more susceptible to radiation effects.

(*j*) Radiation effects with eggs, egg-water, and sperm are graded by dosage of radiation.

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GENETIC EVIDENCE FOR DIPLOID MALES IN *HABROBRACON*.[1]

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The production of males from fertilized eggs in the parasitic wasp, *Habrobracon juglandis* (Ashmead), is discussed in a previous paper (Whiting, Anna R., 1925). It was shown that homozygous orange-eyed females, when crossed to related males with dominant black eyes, produced a few practically sterile black-eyed sons, in addition to the usual number of impaternate orange-eyed males and heterozygous black-eyed daughters. The few daughters of these "patroclinous" or biparental males were black-eyed, almost completely sterile and in many cases abnormal in appearance. It was postulated that a spermatozoön developing independently in the egg cytoplasm and crowding out the egg nucleus might produce a male which, although resembling its diploid sisters, would be haploid. This did not explain why recessive males failed to appear in broods from the reciprocal cross of homozygous black females by orange males; neither did it throw any light on the sterility of the biparental males and of their few daughters.

With the occurrence of new mutations additional facts have been brought out consistent with previous results and throwing further light upon the problem.

A series of quadruple allelomorphs affecting eye color (Whiting, Anna R. and Burton, Raymond H., 1926) has been used in various combinations in breeding experiments to be discussed. These are black (O), light (o^l), orange (o), and ivory (o^i), in decreasing order of dominance. In black, orange, and ivory the ocelli are of the same color as the compound eyes. In light, they are gray while the compound eyes remain black. Distinction from

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[1] The author is indebted to Bussey Institution, Harvard University, for the use of space and equipment, and to the Committee for Research in Problems of Sex of the National Research Council for financial aid in carrying on this work.

type is more easily made in females than in males and is especially clear in light females heterozygous for orange or ivory.

Three pairs of allelomorphs affecting the wings have also been used. Wrinkled (w), recessive to type (W), prevents complete expansion of the wings and often the normal development of legs and antennæ. It is almost completely recessive and somewhat lethal in the homozygous and azygous conditions. Reduced (r), completely recessive to type, reduces the wings, especially the primaries, in size and venation. No overlapping with type occurs. These mutations are discussed at length elsewhere (Whiting, P. W., 1926). Defective (d) (referred to in previous publication as d_{1195}, Whiting, P. W., 1924), like wrinkled, is almost completely recessive but shows some overlapping with type (D). It reduces the length or causes disappearance of the fourth branch of the radius vein (r_4) in 90–95 per cent. of pure stock under standard conditions. Unfortunately reduced and defective cannot always be recognized with certainty in wrinkled, while defective cannot be identified in reduced where the veins are so generally disarranged.

There is no linkage between any of these factors.

Crosses Producing Biparental Males.

Table I. gives summaries of crosses which produce biparental males. Whenever significant differences involving locus D occur they are indicated in the formulæ. The impaternate haploid males arise from unfertilized eggs and resemble their mothers. Where the mothers are heterozygous these males fall into two classes as expected. Females show all the dominant traits of both parents as do the biparental males.

The first cross in the table will serve to illustrate this. Mothers are light, fathers black. All daughters are black, most sons light, the few biparental sons black.

In classes c and e, section I., mothers are defective (d), fathers normal (D). If numbers in these classes be combined it is found that 6 of the 58 biparental males are defective, 10.34 ± 2.70 per cent.; 753 of the 820 impaternate males, 91.83 ± 0.64 per cent.; 46 of the 569 females, 8.08 ± 0.77 per cent. Similarity of percentages in biparental males and females is apparent. The 13 light males produced in classes d and e give still further evidence of biparental inheritance. Their mothers were orange or ivory, fathers light. They resemble their heterozygous sisters as their ocelli are lighter in color than those of males from light stock.

Table I.

Crosses Producing Biparental Males.

Section.	Parents.		Matings	Progeny.		
	♀.	♂.		Bipa-rental ♂♂.	Impa-ternate ♂♂.	♀♀.
I *a*	o^1o^1	O	10	7	156	115
b	oo	O	246	489	11,221	8,436
c	o^1o^1dd	OD	32	55	764	544
d	oo	o^1	8	10	276	326
e	o^io^idd	o^1D	1	3	56	25
f	o^io^i	o	11	32	744	545
II *a*	OOrr	OR	4	8	93	137
b	oorr	oR	4	5	47	54
c	o^io^irr	o^iR	9	9	45	92
III *a*	o^io^irr	o^1R	1	3	4	22
b	o^io^irr	oR	9	5	39	94
c	o^1o^1RR	Or	17	17	392	393
d	ooRR	Or	26	30	758	808
e	o^io^iRR	Or	2	3	119	110
f	OOrr	oR	3	1	16	35
g	OOrr	o^iR	1	5	11	15
h	oorr	o^iR	6	8	82	78
IV *a*	ooddww	ODW	3	5	43	93
b	ooWW	Ow	9	19	643	440
c	ooddWW	ODw	14	29	660	404
d	o^io^iddWW	ODw	19	17	637	360
e	ooWw	OW	1	13	79	77
V *a*	ooWWrr	OwR	5	7	63	83
b	ooDDWWrr	OdwR	3	3	6	26
c	ooddWWrr	o^iDwR	3	4	17	70
d	oo^iWWrr	owR	3	3	41	48
Total			450	790	17,012	13,430

In section II. are data on crosses where parents differ in wing character, locus R, but are similar in eye color. Biparental males can be readily recognized by their non-reduced wings. These are of interest since they show that the chromosome carrying R acts in the same manner as those containing O and D.

In section III. are given summaries of crosses involving differences in O and R. Whether one or two recessive factors are present in the mother the biparental males show both dominant characters.

Section IV., deals with crosses involving differences in the O and W loci, and in some cases in D. Cross a, ooddww females by ODW males, gave 5 black-eyed males 1 of which was wrinkled. All had normal venation. Since homozygous wrinkled females have bred true in all cases tested by the author there is little doubt that the W is contributed by the father. All orange sons were wrinkled, practically all that could be identified were defective, and all black daughters normal as to wings.

Wrinkled sometimes occurs as a result of accident of growth. Since biparental males are abnormal in so many ways, as for example in their rather common habit of pupating without spinning a cocoon, their wings might have a greater chance to be wrinkled, thereby increasing percentage of wrinkled in this class.

Cross c, section IV., ooddWW female by ODw male, is of importance. A dominant factor affecting the wings is contributed by each parent. Of the 29 black non-defective sons, 25 were non-wrinkled while 4 had slightly wrinkled wings, a condition parallel with that found in the sisters except that percentage of wrinkled is higher. There was one wrinkled among the 660 orange males and 3 wrinkled among the 404 black females. Class d resembles this except that ivory females were used instead of orange. Results are similar. Nineteen matings gave 17 black males, 1 with wrinkled wings and 1 with defective venation, 637 ivory males, practically all defective, and 360 black females, 1 with wrinkled wings.

In section V. are types of crosses involving differences in O, W, R, and in two cases D. In class a, ooWWrr females by OwR males, there were produced 7 black, non-wrinkled, non-reduced males and 63 orange non-wrinkled reduced males. The females were like the biparental males except that 1 defective appeared. In class b the four differences are involved, two dominant factors contributed by each parent. The 3 biparental males were entirely dominant, the orange sons non-wrinkled and reduced, the daughters dominant except for two defective individuals. Wings of biparental males show three dominants, two from the mother, one from the father.

Class c is likewise of special interest. Recessives d and r are contributed by the mother, o^i and w by the father. Biparental males are orange, non-defective (with one exception slightly so), non-wrinkled, and non-reduced. Here again the wings of biparental males show three dominant characters, two from the father and one from the mother. In class d three females heterozygous for o and o^i, homozygous for W and r, are mated to owR males. All ivory males have reduced wings while among the orange males are the biparental males easily recognized by their non-reduced wings.

In all, four hundred and eighteen matings were made where female parent had recessive eye color, male dominant. 747 biparental males were produced in addition to 16,660 impaternate males and 12,901 females.

One hundred and thirty-five matings of defective females by non-defective males gave 307 biparental males, 6,325 impaternate males and 3,816 females. Defectives were recorded in one hundred and two of these matings. 30 defectives were found among 269 biparental males, 11.15 ± 1.29 per cent.; 4,744 among 5,159 impaternate males, 91.95 ± 0.25 per cent.; 175 among 3,085 females, 5.67 ± 2.81 per cent.

Three matings of ww females by W males gave 5 biparental males, 1 wrinkled, 43 impaternate males, all wrinkled, and 93 normal females.

Fifty-one matings of rr females by R males gave 61 biparental males, 462 impaternate males and 754 normal females.

Tests of Biparental Males.

Table II. gives summary of tests of biparental males. Whenever possible they were tested by mating to homozygous recessive females. Individual males were often mated to several females. Since they produce but few daughters the results of a large amount of work seem meager.

Two hundred and forty-two males were tested. One hundred and ninety-seven or 81.40 ± 1.69 per cent. were found to be sterile. Of these, one hundred and thirty-nine were tested once, forty-five twice, six three times, four four times, two five times, and one seven times, in all two hundred and eighty matings resulting in 23,089 sons, no daughters.

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TABLE II.

TESTS OF BIPARENTAL MALES.

Probable Formulæ of ♂♂.	Source (See Table I).	Sterile ♂♂.	Sons of Mates.	Fertile ♂♂.	Daughters.	Sons of Mates.
Oo^1	I *a*	4	396	0		
Oo	*b*	145	18,692	31	104	3,769
Oo^iDd	*c*	4	145	0		
o^1o	*d*	2	56	1	5	38
o^1o^iDd	*e*	1	72	0		
oo^i	*f*	7	654	3	13	750
OORr	II *a*	1	159	1	21	66
ooRr	*b*	3	265	0		
o^io^iRr	*c*	7	346	1	1	25
oo^iRr	III *b*	1	55	1	6	9
Oo^1Rr	*c*	5	137	2	6	160
Oo^iRr	*g*	1	56	2	25	816
oo^iRr	*h*	4	323	3	5	296
OoDdWw	IV *a*	5	1,010	0		
OoDdWw	*c*	4	705	0		
OoWW(w)	*e*	3	18	0		
Total		197	23,089	45	186	5,929

Of the forty-five fertile males twenty-six were tested once each, seven twice each, four three times, two four times, three five times, one seven times, and two ten times, one hundred and two matings. Altogether these matings gave only 186 daughters among the 5,929 sons of the females, an average of 3.135 daughters for each fertile biparental male. Seven matings of one male made at two day intervals resulted in 21 daughters, 15 of these in one mating. Another male mated ten times at two-day intervals gave in five matings 21 daughters. These are the most prolific by a rather wide margin. Results indicate that more daughters could be obtained from biparental males by making repeated tests.

With two exceptions all biparental males produced daughters showing the dominant characters like themselves. They therefore breed like haplonts. This was true of the D, O, and R loci irrespective of the side from which the factors came. Although nine biparental males carrying w were tested by crossing to ww females, some of them several times, no daughters were obtained among the 1,733 sons, all wrinkled like their mothers.

Daughters of Biparental Males.

Of the two exceptional biparental males mentioned above one occurred in a mating of ivory defective female by orange defective male. He had orange eyes and defective wings as would be expected. His mate was of the same genetic constitution as his mother. In the first culture bottle appeared 2 orange females, 1 with abnormal abdomen which died within the cocoon, the other sterile. In the fourth bottle was found an ivory defective female. She was fairly fertile. In the first culture bottle she laid over thirty eggs, one of which hatched into an ivory female. In the second bottle there was likewise a high mortality of eggs but 9 ivory males and 5 ivory females ultimately emerged. Her daughter from bottle a gave 49 males, another which lived but a short time gave 10 males. There are three possible explanations for the appearance of this ivory female. Her mother may not have been virgin when mated. This is improbable since great care was exercised in this matter and many more ivory females would be expected early in the life of the mother if she had mated with an ivory brother. The female may have been produced from an unfertilized egg, a phenomenon which has occurred but rarely in *Habrobracon,* or the male may have produced a spermatozoön not carrying the chromosome containing the o factor.

The comparatively late appearance of this female serves as an argument for the second explanation since daughters of biparental males usually appear in the first or second culture bottles. On the other hand the high mortality of her eggs might indicate that she is the daughter of the biparental male since the few females obtained from unfertilized eggs have proved highly fertile.

The second exceptional case was found early in the work by P. W. Whiting. It has not been previously discussed in detail. A male of type stock 1 was crossed with female of orange defective stock 3. 118 orange sons showed defect typical of stock 3. Black daughters were 15 normal, 1 defective; black sons were 3 normal, 1 defective, the defective among the black being due to irregular dominance. One of these normal black males was mated to three orange females by each of which he produced a single daughter.

From one cross there were produced 30 orange males and in vial b a single black-eyed female of small size and with asymmetry of ventral abdominal sclerites. She produced only one larva which died. Whether she posessed D or d is not known. Her morphological abnormality and near sterility are comparable with such conditions in daughters of biparental males transmitting only dominant traits.

From another cross there were produced 92 orange males and in vial c a single orange female. She developed from a naked pupa and had asymmetrical sternites. She appeared normal in internal morphology and histology and produced 3 orange sons, 1 normal and 2 defective. Since her mother was of normal orange stock the occurrence of these indicates that she had received d from her father. Like many daughters of other biparental males she was of abnormal appearance, but unlike them she was somewhat fertile.

From the third cross there were produced 136 orange males and, in vial a, a single orange female. This female was normal in appearance and produced 71 offspring, males 13 normal, 16 defective, and females 23 normal and 19 defective. The defectives were due to the fact that this female's mother had d. Normal venation was isolated in later generations.

This biparental male had, therefore, in addition to black-bearing spermatozoa, two types of orange, od, the maternal combination, and oD, a recombination type. He is the only male found which breeds like a heterozygote. He and his daughters are not included in the following summary.

Of the 186 dominant daughters of biparental males only 121 were sufficiently normal to test. Most of these laid eggs which failed to hatch. Some lived for several days, stung the host caterpillars but laid no eggs. One gave a normal black male which proved sterile; one produced a black-eyed female pupa which died in the cocoon; one gave a morbid larva which died young and an abnormal female pupa with black eyes found dead in the cocoon; and another an abnormal pupa of uncertain eye-color and sex, and a fifth *five* larvæ which died and a female pupa of uncertain eye-color. The dominant daughters have so far given only dominant offspring.

Morphological Abnormalities in Biparental Males and their Daughters.

Physical defects are rather common in biparental males and their daughters. These include abnormal sclerites in abdomen, defects in antennæ, abnormal legs, incomplete digestive tract, abnormal thorax, and genitalia. Among the 790 biparental males were found 41 or 5.19 ± 0.53 per cent. abnormal. Also there were 61 individuals that did not spin cocoons (called naked pupæ) 7.72 ± 0.64 per cent. This stands in contrast to conditions in impaternate males where there were but 24 abnormal and 83 naked pupæ among 17,111 normal, 0.14 ± 0.02 per cent. and 0.48 ± 0.04 per cent. respectively. Among the 13,430 sisters of biparental males there were 82 abnormal and 57 naked pupæ, 0.61 ± 0.04 and 0.42 ± 0.04 per cent. respectively.

In the daughters of biparental males abnormalities are often more extreme and present in a much higher percentage. 68 freaks and 23 naked pupæ appeared in addition to 95 normal. Percentage of freaks is 36.56 ± 2.38, of naked pupæ 12.36 ± 1.63. Brothers of these females showed 13 freaks and 2 naked pupæ to 5,914 normal, 0.22 ± 0.04 and 0.03 ± 0.02 per cents.

Summary.

1. Four allelomorphs affecting eye color and three pairs of allelomorphs affecting wing form and venation, none linked, are studied from the point of view of the method of their inheritance by biparental males in *Habrobracon juglandis* (Ashmead).

2. A female homozygous for one or more recessive factors when crossed to a male carrying allelomorphs to these factors produces, in addition to recessive haploid sons and dominant diploid daughters, sons which have all dominant characters like their sisters.

3. In crosses where females are homozygous for some recessive and some dominant factors and males possess allelomorphs the biparental sons are entirely dominant, showing that they have some factors from each parent.

4. When three of these factors affect one structure (the wing in this case) if one recessive and two dominants are contributed by one parent, their allelomorphs by the other, this structure in biparental males shows all the dominant characters.

5. From these results it is concluded that biparental males are diploid (Whiting, P. W. and Whiting, Anna R., 1925) at least for the four chromosomes that can be identified genetically.

6. Biparental males and their daughters are often abnormal in appearance and usually sterile or nearly so. When fertile they breed as dominants (with one, and possibly two, exceptions noted above).

DISCUSSION.

When different types of crosses are made between inbred related stocks and results summarized it has been found that definite relationships exist between various percentages derived from these summaries.

Percentage of males among biparental offspring, (previously called percentage of patrocliny), $\frac{\text{biparental } ♂♂ \times 100}{\text{biparental } ♂♂ + ♀♀}$, is negatively correlated with percentage of females, $\frac{♀♀ \times 100}{\text{total,}}$, and with percentage of total biparentals, $\frac{\text{biparental } ♂♂ + ♀♀ \times 100}{\text{total}}$. In other words when a type of cross results in a high percentage of males among biparentals there is a low percentage of females and of total offspring from fertilized eggs. The female percentage is lower than it would be if the decrease were due only to the transformation of some fertilized eggs into biparental males. This indicates that there is a mortality of fertilized eggs in these crosses directly correlated with number of biparentals which are males. Types of crosses that produce no biparental males have the highest percentage of females.

The question as to why these individuals are males cannot be answered at this point. Dr. Castle has suggested that they may correspond to the intersexes which Goldschmidt gets in *Lymantria* (Goldschmidt, Richard, 1927). Some of the facts support this and it may be that the presence of certain genetic factors in some individuals causes sex reversal. No gradations have been observed in *Habrobracon*. The biparental males have all been completely male in external and internal morphology and in reactions. The high mortality suggested above may be due to intergrades that cannot survive in this species. In *Lymantria* the intergrades occur as a result of wide crosses while in *Habrobracon* these males come from crosses of related stocks only.

Non-disjunction may also be considered a possibility. Biparental males may be diploid for all chromosomes save one, the sex chromosome, and thus be males although resembling their sisters in appearance.

Unfortunately *Habrobracon,* like the Hymenoptera in general, is not ideal for cytological study. Haploid number of chromosomes seems to be eleven and they are extremely minute. Nachtsheim has demonstrated (Nachtsheim, H., 1913) that in the honey bee chromosomes frequently fractionate so that somatic counts vary considerably being various multiples of the haploid number. The author finds indications of this in another form now under observation so that a very careful study and numerous counts are necessary before conclusions can be drawn.

In spermatogenesis of biparental males the first maturation division is abortive, the second apparently equational as in normal haploid males. This process may result in diploid spermatozoa which when united to recessive eggs would give only dominant triploid offspring. The high percentage of physical abnormalities in daughters of biparental males and their sterility may be due to their triploidy. They possess ovaries and ova normal in general appearance.

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CARBON DIOXIDE AS A NARCOTIC AGENT.

I. The Effect of Carbon Dioxide upon the Fertilized Egg of *Arbacia*.

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The narcosis which may be produced by carbon dioxide seems to have long been known. Pliny the Elder, in his Natural History, remarked that the marble from Memphis, when ground up and used as a liniment with vinegar, had the virtue of rendering insensible parts of the body to be cut or cauterized. In modern times the value of carbon dioxide as an analgesic was recognized soon after Black's discovery of "fixed air," and in 1788 we find Percival recommending "fixed air" for the relief of painful ulcers. Later it was employed as a local anesthetic by Ingenhousz,[1] Beddoes, Simpson, Follin, Brown-Séquard,[2] Gellé,[2] and others. In 1828, eighteen years before Morton's demonstration of ether anesthesia, Hickman[3] appears to have suggested that general anesthesia be induced by inhalations of carbon dioxide, and it was thus used later in the century by Ozanam and by Gréhant, although never extensively. Ozanam seems to have been impressed by the promptness of recovery from the anesthesia produced in this way. At one time it was thought by some that natural sleep and hibernation were the effects of the accumulation of carbon dioxide. This view has been abandoned, but Kidd (1914) has recently with more plausibility suggested a similar explanation for the dormancy of seeds. The work of Cohn (1918) also indicates the possibility that carbon dioxide may play an important part in keeping the spermatozoa of *Arbacia* inactive until their discharge into sea water.

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[1] Cited by Herpin (1864).

[2] Cited by Dastre (1890).

[3] Cited by Simpson (1856).

Inasmuch as carbon dioxide is a normal product of metabolism, an understanding of its characteristic effects on living cells and the mechanism by which they are produced is of importance. Unfortunately, most of the studies previously made on its action have been concerned with entire organisms where numerous complicating factors prevent the separation of its more characteristic effects from others of a less fundamental nature. It is the object of the present paper to study in a quantitative way certain aspects of the narcotic effects of carbon dioxide on single cells—the developing egg cells of *Arbacia*—where complicating factors are reduced to a minimum. In particular, an attempt has been made to determine the extent to which such effects are reversible. A second paper [1] deals similarly with a simple tissue—the striated muscle of the frog—and studies upon ciliated epithelium are to be reported elsewhere.

For quantitative work, the developing egg of *Arbacia* proves very satisfactory. Since favorable material shows about 100 per cent. division under normal conditions, any delay in the occurrence of the first cleavage may be measured and used as a suitable quantitative criterion for narcotic or toxic effects upon the activity of the cell. Smith and Clowes (1924) have studied from a somewhat different point of view from that of the present paper the effects of carbon dioxide upon the cleavage of echinoderm eggs and have found that development is inhibited by CO_2 at pH values which in its absence are without effect upon cleavage. The present experiments differ in at least two respects from those of Smith and Clowes. In the first place, they have to do with exposures of varying lengths *followed by a return to normal sea water* to permit a determination of the extent to which the effects produced are reversible. In the second place, a different method has been used for the quantitative measurement of the degree of retardation of the developmental processes. Instead of measuring the total number of cell divisions secured in a given time irrespective of whether they be the first, second, or third, the quantity here measured has been the time required for the first cleavage in each case. Furthermore, this has been done in such a way as to take into account not merely the mean time for all of the eggs but, approximately at least, the time for each individual egg. It is believed that data of this sort, while more troublesome to secure, are theoretically more significant and have a wider range of usefulness than those obtained by the other method.

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[1] *Amer. Journ. Physiol.*, LXXXII., 241.

METHOD.

Sea water, saturated with CO_2 from a Kipp generator, was used directly or was diluted as desired with oxygen-saturated sea water, with nitrogen-saturated sea water, or with ordinary sea water by siphoning together appropriate amounts of these solutions. It was then immediately siphoned into 75 cc. glass-stoppered bottles containing a few drops of a concentrated suspension of the newly fertilized eggs of *Arbacia punctulata.* As soon as the bottles were completely filled, they were tightly stoppered, shaken briefly, and placed in a bath of running sea water, which, with a few exceptions, varied in temperature not more than 0.5° C. for the duration of each experiment. For all the experiments during one season the range of temperature was 18.4° C. to 22.4° C., with a mean value of 20.3° C. In order to prevent a change in the solubility of the dissolved gases, it was deemed important always to have the CO_2-containing solutions at the temperature of the water-bath. Care was also taken to begin all the exposures of any one series as nearly simultaneously as possible, since a number of experiments not reported here seemed to indicate that sensitiveness to CO_2 may vary prior to the appearance of the first cleavage. In many of the experiments the bottles were inverted at five minute intervals to keep the contents well mixed, but other experiments in which this precaution was less rigorously observed gave essentially the same results.

At the time of setting up an experiment, samples of the solutions used were taken for estimations of the pH and dissolved oxygen. The former were immediately determined with the use of phenol red, brom thymol blue, brom cresol purple, and methyl red as indicators; the samples for the latter were kept at a constant temperature until Winkler determinations could be made. The results of the Winkler determinations are expressed as cc. of oxygen per liter. Otherwise, the oxygen content is stated, as is the carbon dioxide content, in terms of percentage saturation. Assuming the applicability of Henry's law to gases in solution, it may be said that when 20 cc. of oxygen-saturated sea water is added to 80 cc. of CO_2-saturated sea water, the resulting solution contains CO_2 at 80 per cent. of saturation value and oxygen at 20 per cent. of saturation value. Since in adding the saturated solutions to the eggs they must experience a small interchange of gases with the air, a solution which was initially free of oxygen is referred to as having a trace of oxygen, while a CO_2-saturated solution is represented as having a CO_2 content of "100 —" per cent. Wherever the tension of CO_2 is expressed in mm. Hg the value given is merely an approximate one, calculated for purposes of comparison with the work of other investigators on the assumption that the tension of carbon dioxide in a saturated solution is 760 mm., minus the vapor pressure of water at the temperature in question.

As already mentioned, the reversibility of the effects of CO_2 was determined by returning the eggs to sea water for development after the desired periods of exposure. Following a rinsing in sea water, the eggs were placed in small Pyrex beakers containing sea water to a depth of about 1.5 cm. Samples of the eggs were removed from the beakers at various times and were preserved for subsequent observation by the addition of a weak solution of formalin in sea water. The fixed eggs were placed in a large hanging drop where, if free from debris, they tended to settle in rows, which simplified the task of determining the percentages of eggs which had undergone the first cleavage. These values, found from time to time after a given exposure, give, when plotted against the minutes after fertilization, a curve which will be referred to as the cleavage curve for that particular exposure. The characteristic S-shape of this curve is related to the variability of the eggs themselves in the manner discussed by Loeb and Northrop (1917) and by Brooks (1918). The time required for cleavage in 50 per cent. of the eggs has in these experiments been used as the most convenient criterion of the cleavage rate, but other percentages could equally well be compared; the time in question can, of course, be readily found by interpolation from the cleavage curve.

RESULTS.

Before discussing the typical effects of carbon dioxide, it is necessary to rule out the possibility that oxygen lack might be a contributing factor in the results produced, since the method used in saturating the sea water with carbon dioxide causes at the same time a removal of oxygen. Although a complete lack of oxygen has been shown to stop the cleavage process in sea urchin eggs (E. B. Harvey, 1927), the present work indicates that even with an extensive reduction in oxygen tension cleavage is able to continue—and at a rate but little slower than normal. The results of the thirty minute exposures to low tensions of this gas, representing but 14 per cent. to 18 per cent. of those available for the controls, are given in Table I. and show that the cleavage time under these conditions was delayed but a few minutes.

TABLE I.

THE EFFECT UPON CLEAVAGE OF THIRTY MINUTE EXPOSURES TO LOW OXYGEN TENSIONS.

Solution.	cc. of Oxygen per Liter.	Minutes Required for 50 per cent. Cleavage.
Sea water	5.6	60.5
Nitrogen-saturated sea water	0.76	68.5
Sea water	5.48	62.5
Nitrogen-saturated sea water	0.97	64.2

A direct comparison of the effects of low oxygen tension and of high carbon dioxide tension has been made in another experiment, in which each of four portions of egg suspension was exposed for thirty minutes to one of the following solutions:

	pH.	cc. of Oxygen per Liter.
1. Sea water	—	5.15
2. Sea water + oxygen + CO_2 (60% saturated)	5.3	5.56
3. Sea water + nitrogen + CO_2 (60% saturated)	5.3	1.26
4. Sea water, saturated with nitrogen	—	0.55

It will be evident that solutions 3 and 4 were low in oxygen as compared with solutions 1 and 2, while solutions 2 and 3 were high in CO_2 as compared with solutions 1 and 4. The first cleavage appeared as shown in Fig. 1, where it will be seen that the shortest cleavage time occurred with the sea water control, represented by Curve 1. A lowering of the oxygen tension to slightly over one tenth of the normal value retarded the cleavage time but 4 minutes

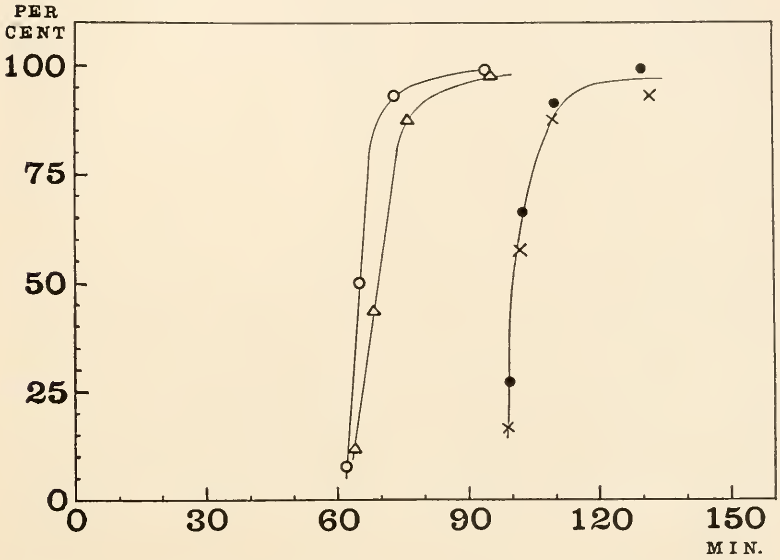

Fig. 1. The relative effectiveness of low oxygen tensions and high CO_2 tensions in delaying the first cleavage of *Arbacia* eggs. Exposures were of thirty minutes' duration. Temperature, 19.8°–20.2°. Abscissa = time, in minutes, after insemination. Ordinate = percentage of eggs showing the first cleavage.

	Symbol.	cc. Oxygen. per Liter.	CO_2.
Curve 1	○ Circles	5.15	
Curve 2	× Crosses	5.56	60%
Curce 3	● Dots	1.26	60%
Curve 4	△ Triangles	0.55	

(Curve 4). On the other hand, an increase of the CO_2 content to give pH of 5.3 was sufficiently great to retard the cleavage time 36 minutes, irrespective of the oxygen present, since a single curve serves to represent cleavage in the two solutions which, although showing more than a 4 : 1 difference in oxygen content, had the same carbon dioxide content. Since from such experiments as the foregoing the cleavage process is seen to be but little affected by oxygen deficiency over a wide range, the more striking effects observed with CO_2, about to be described in more detail, may justly be attributed to an action of carbon dioxide in which incidental oxygen lack plays little or no part.

Such being the case, the solutions used in the subsequent experiments were simply mixed in a large graduated cylinder and immediately added to the eggs. Siphons were not used nor were Winkler determinations made. Speed in setting up the experiment was important, and in cases where a considerable number of bottles were to be filled, this method had the advantage of expediency. Where the sea water used was saturated with CO_2 (i.e. "100 —" per cent. CO_2, with a trace of oxygen) it seems probable that sufficient oxygen must have entered the solution from the air to prevent any retardation of cleavage from oxygen lack, since a 4 : 1 mixture of CO_2-saturated and oxygen-saturated sea water (*i.e.* 80 per cent. CO_2 and 20 per cent. oxygen) gave practically the same result. In fact, the results following "100 —" per cent. CO_2 were very similar to those of various tensions down to as low as about 30 or 40 per cent. CO_2. Inasmuch as most of the work here reported is not concerned with more than a semi-quantitative estimate of the gases, it is believed that the method is sufficiently accurate.

In striking contrast to the extensive diminution in available oxygen which the eggs seem to tolerate is the effect upon cleavage of even very small amounts of carbon dioxide. The repression of cleavage which occurs when, in the laboratory, the eggs are subjected to overcrowding is a familiar example of the effects which may be produced simply by the CO_2 which arises from the metabolism of the cells themselves. Experimentally, sea water containing as little CO_2 as 10 per cent. of saturation value was found, after an exposure of twenty minutes to delay cleavage twelve and one half minutes, while exposures of equal length to 30 per cent. and 40 per cent. CO_2 were found to delay cleavage a longer time—about twenty-three minutes. Apparently this latter value of 40 per cent. saturation or 300 mm. Hg represents practically a complete suppression of cleavage since it is approximately the same value as is obtained with higher tensions.

TABLE II.

PERCENTAGE OF EGGS CLEAVED DURING EXPOSURE TO CO_2.

Experiment.	No. 130.	No. 131.	No. 129.	No. 120.
Temperature	22.3°	21.7°–22.1°	21.3°–22.4°	21.2°–21.6°
Per cent. CO_2	15%	20%	30%	80%
pH	*6.25*	*6.2*	*5.95*	*5.2*
Exposures (in minutes):				
80′	10.9%	0%		
90′	27.7%			
100′		1%	0%	
120′			0%	
140′	56.8%			
160′			0%	
200′			6.8%	
300′			43.8%	
640′				0%

It is evident from Table II. that with less than 30 per cent. saturation the repression of cleavage is not complete, but only partial. At a value of 30 per cent., repression is almost complete, since only 7 per cent. and 44 per cent. of the eggs were able to cleave in three and one third and five hours respectively. It will be shown later in the paper that the effects of 30 per cent. and 40 per cent. CO_2 are so similar to those produced by greater amounts, that it is reasonable to suppose that at a saturation of 40 per cent. and over, representing a tension of 300 mm. Hg or more, the suppression of cleavage is practically complete. Therefore not only can very small amounts of CO_2 in the surrounding medium cause an appreciable delay in cleavage, but also its maximum action in suppressing cleavage is approached at values far below saturation.

Notwithstanding the prompt and extensive check upon cleavage which carbon dioxide produces, the reversibility of its effects upon returning the eggs to sea water serves to indicate that the action has been of a narcotic rather than of a purely toxic nature. After exposures of twenty minutes to sea water practically saturated with CO_2 there are usually no abnormalities in cleavage, and larvæ develop which show little or no difference from the normal controls. Following longer exposures, abnormalities appear, although they are relatively few in number with exposures of less than an hour. In spite of the presence of many abnormally cleaved eggs, a few ciliated larvæ have been found to develop after an exposure of two and one half hours, and the first cleavage has made its appearance in 95 per cent. to 100 per cent. of the eggs subjected to very nearly 100 per cent. CO_2 for this length of time, though relatively few normal larvæ were obtainable after such long exposures. In one experiment—that illustrated in Table III.—exposure of the eggs to 80 per cent. CO_2 for over ten hours still permitted reversibility of the cleavage process to the extent that, six hours after the eggs had been returned to sea water, 88 per cent. were found to have divided, although development was very abnormal and went no farther than the first few cleavage stages. Apparently the process of nuclear division is an extremely powerful one and, as has been observed by others, can persist even when the cell itself is unable to divide.

Fig. 2 is a typical illustration of the series of cleavage curves which are obtained with various lengths of exposure to a relatively high tension of carbon dioxide. It will be observed that the first cleavage ultimately occurs in practically all of the eggs, even after prolonged exposures. The relation of the exposure time to the total retardation is a matter of some importance in indicating the nature of the observed effects. If the narcosis produced by carbon dioxide were complete and were followed by instantaneous recovery the resulting retardation of cleavage, as compared with normal controls, should be exactly equal to the time of exposure. Incomplete narcosis, on the other hand, would tend to shorten, and a more gradual recovery, to lengthen, the period of retardation. Theoretically, a combination of these two effects might conceivably, under a given set of conditions, result in a net retardation exactly equal to the period of the exposure, but it is believed that such a balancing of effects could not account for the results about to be described. Since with all tensions above approximately 30 per cent. saturation the relation of time of exposure to total retardation is essentially the same, it is difficult to imagine that the CO_2 tension should, above this point, either be without effect on a measurable rate of development or that a decrease in this rate should always be followed by a correspondingly increased rate of recovery. The reasonable interpretation of the facts is that development is practically suppressed by the higher tensions of CO_2. The correctness of this view is also indicated by the fact that cleavage was not obtained during prolonged exposures to such solutions.

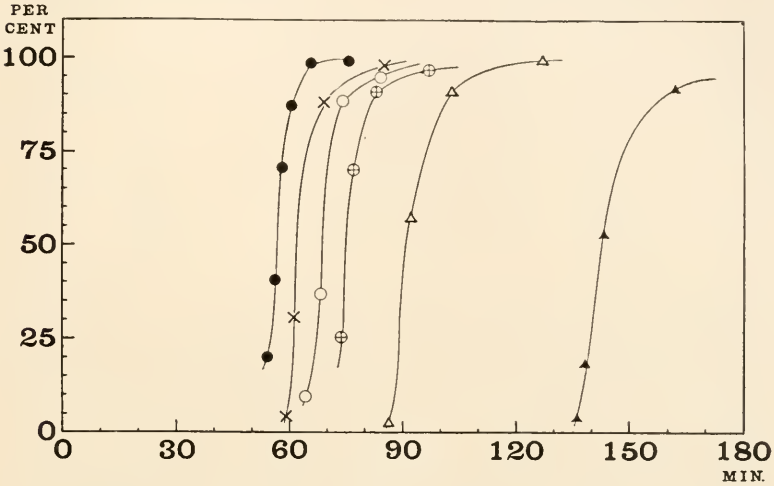

FIG. 2. The times required for the appearance of the first cleavage of *Arbacia* eggs following various lengths of exposure to sea water 80 per cent. saturated with CO_2. Temperature, 21.2°–21.6°. Abscissa = time, in minutes, after insemination. Ordinate = percentage of eggs showing the first cleavage.

Symbol.	Length of Exposure.
● Dots	0 minutes
× Crosses	5 minutes
○ Circles	10 minutes
⊕ Crosses in circles	20 minutes
△ Hollow Triangles	35 (?) minutes
▲ Solid Triangles	80 minutes

For each of the six curves the final value, omitted from lack of space, was 100 per cent., except in the case of the twenty minutes exposure, where it was 98 per cent. There have also been omitted the cleavage curves following exposures of 160, 320, and 640 minutes, which ultimately showed cleavage in 97 per cent., 99 per cent., and 88 per cent. of the eggs respectively.

Table III. gives the delay in 50 per cent. cleavage corresponding to each cleavage curve of the complete series.

The relation between time of exposure and retardation of development is shown for a typical experiment in Table III., the values for 50 per cent. cleavage having been taken from the curves in Fig. 2.

TABLE III.

THE DELAY IN 50 PER CENT. CLEAVAGE CAUSED BY VARIOUS EXPOSURES TO 80 PER CENT. CO_2.

(Experiment illustrated in Fig. 2.)

Exposure Time (in Minutes).	Delay in 50 per cent. Cleavage (in Minutes).
0	—
5′	5′
10′	12′
20′	18′
35′ (?)	34′
80′	86′
160′	186′
320′	370′
640′	750′

It will be observed that with the shorter exposures the recovery of the cleavage process may occur with great rapidity, the delay being but little more than the time corresponding to the period of exposure. Consequently, it has very often been found possible, in performing experiments, to predict the beginning of cleavage with a fair degree of accuracy simply by adding the exposure time to that required for the beginning of cleavage in the normal. As exposures become longer, the greater discrepancy between the exposure time and the delay of cleavage is wholly in one direction—that of prolonging the cleavage time. A further analysis of the data shows even in this respect a simple relation which may best be brought out by plotting the time required for 50 per cent. cleavage against the time of exposure, as has been done in Fig. 3. It will be noticed that straight lines may be drawn through the points representing any given experiment. Those indicated in the figure have been calculated by the method of least squares, with the result that the slopes of all the lines are approximately equal. All may be represented fairly accurately by the equation

$$y = a + 1.18x,$$

where $a =$ the cleavage time of the eggs in the absence of CO_2; $x =$ the time of exposure, and $y =$ the cleavage time of the exposed eggs. Further data are given in Table IV.

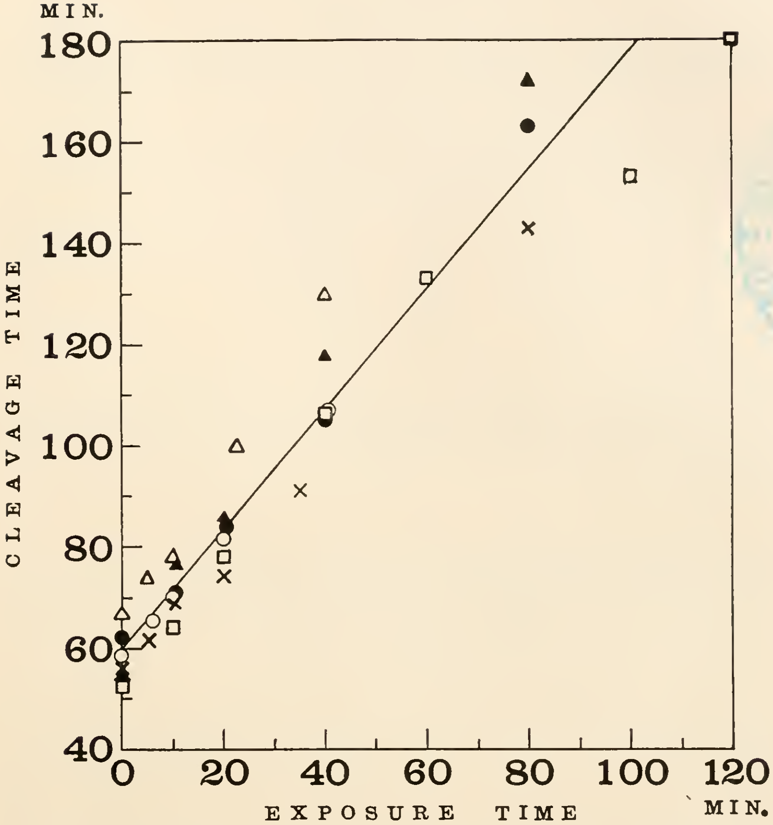

FIG. 3. The relation between the time required for 50 per cent. cleavage of *Arbacia* eggs and the duration of exposure to CO_2 at values from 30 per cent. saturation to practically complete saturation. Abscissa = minutes of exposure to CO_2. Ordinate = minutes required for cleavage in 50 per cent. of the eggs. Details are given in Table IV.

Symbol.	Experiment.
○ Circles	No. 119
× Crosses	No. 120
△ Hollow Triangles	No. 121
▲ Solid Triangles	No. 122
● Dots	No. 128
□ Squares	No. 129

(From lack of space, several values for exposures of over 120 minutes have not been included. These values were, however, used in calculating the equations the average of which—$y = 60.2 + 1.18x$—gives the straight line shown in this figure.) The slope of the curve would be unity, if recovery of the eggs were instantaneous upon their removal from CO_2.

TABLE IV.

EQUATIONS FOR THE RETARDATION OF 50 PER CENT. CLEAVAGE IN *Arbacia* EGGS IN SEA WATER 30 PER CENT. TO "100 —" PER CENT. SATURATED WITH CO_2.

Experiment.	CO_2 Content.	Oxygen Content.	Temperature.	Intercept.	Slope.
No. 119.....	"100 —"%	Trace	20.6°–21.2°	58.3	1.192
No. 120.....	80%	20%	21.2°–21.6°	52.6	1.182
No. 121.....	"100 —"%	Trace	20.4°–20.7°	71.	1.191
No. 122.....	"100 —"%	Trace	20.7°–20.8°	65.3	1.194
No. 128.....	40%	20%	20.8°–21.3°	59.35	1.23
No. 129.....	30%	20%	21.3°–22.4°	54.66	1.07
Mean Values.				60.2	1.18

(A preliminary experiment which showed a slope of 1.7 has been omitted from the average since this value differs widely from those subsequently obtained.)

The numerical value—1.18—of the slope of the lines in Fig. 3 shows that the retardation in cleavage produced by a given exposure is nearly, though not exactly, equal to the time of exposure. A value of unity would indicate exact equality. The fact that the slopes of the various lines are nearly the same is an indication of the general similarity, already mentioned, of the CO_2 effects at all tensions above 30 per cent. of saturation. The different values of the intercepts are without significance in this connection, since they represent merely the normal time of cleavage in those eggs which were not exposed to carbon dioxide.

SUMMARY.

1. The first cleavage of the fertilized eggs of *Arbacia* is entirely suppressed, or practically so, in the presence of amounts of carbon dioxide greater than those corresponding to a 40 per cent. saturation of sea water or a tension of approximately 300 mm. Hg. In the presence of smaller amounts of carbon dioxide cleavage is possible, but is greatly delayed.

2. Since a very considerable oxygen deficiency causes only a slight delay in the cleavage process, the factor of oxygen lack is probably a negligible one in the results here described.

3. The effects of a complete suppression of the cleavage process

in sea water practically saturated with carbon dioxide are readily reversible up to exposures of twenty minutes. Beyond that point abnormalities may appear, though after exposures of two and one half hours 95 to 100 per cent. of the eggs ultimately divide.

4. The after effects of exposures of moderate length to carbon dioxide are comparatively slight, the delay in the first cleavage being only a little greater than the actual time of exposure. Mathematically, the relation

$$y = a + 1.18x$$

(where $a =$ normal cleavage time; $x =$ time of exposure; and y $=$ cleavage time of the exposed eggs) has been found to describe fairly accurately the results obtained at 21.4° (± 1.°) with sea water from 30 per cent. saturation to almost complete saturation.

I am glad to have this opportunity to express my gratitude to Dr. M. H. Jacobs for his suggestion of this problem and for his continued interest in the progress of the work.

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ORIGIN AND DESCRIPTION OF BRISTLE IN *DROSOPHILA MELANOGASTER.*

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Origin and Description of Bristle in *D. melanogaster.*

In an experiment designed to test the combined effect of temperature and the genes linked with Curly upon crossing-over in the third linkage group, Dichæte females were mated with sepia spineless sooty rough Curly males. Among the 163 offspring of such a mating there appeared on April 11, 1925, two Dichæte females with the bristles on the head and thorax shorter (1/2 to 3/4 the length of corresponding bristles on the wild type) and slightly thicker than those in the wild type and slightly irregular in outline with a tendency to be twisted and truncate. The new character is called Bristle in the following account (Symbol Bl.). The fact that only two unusual females were found among so many made it seem improbable that the character difference depended upon a recessive or a sex-linked factor difference. Thus by exclusion, it was supposed that the mutant gene was dominant. Further the two individuals probably owed their appearance to a single mutation which may have occurred either in the late oogonial divisions of the female, or, in the spermatocyte or late spermatogonial divisions of the male parent. Since the male carried the dominant Curly and the female the dominant Dichæte it is more probable that the mutation occurred in the female since both individuals were not Curly.

These two females were mated with wild type males and their offspring (which were not counted) included Dichæte, Bristle, Dichæte Bristle and wild type flies. A number of the female Dichæte Bristle offspring were mated separately with homozygous Lobe males. Half the offspring were Bristle which supported the assumption that Bristle was dominant; half the Bristle individuals were also Dichæte indicating that D and Bl are probably in different linkage groups (Table I.).

F_1 Dichæte Bristle Lobe males were mated with wild type females and F_1 Dichæte Bristle Lobe females were mated with wild type males. The male black-crosses showed no recombination of Bl and L so that Bl is in the second linkage group. The female back-crosses gave a cross-over value of 18.1 per cent. between L and Bl (Table I.).

TABLE I.

P_1 DICHÆTE BRISTLE ♀ × LOBE ♂.

L.	D L.	Bl L.	D Bl L.
158	146	142	134

B. C., F_1 DICHÆTE BRISTLE LOBE ♂ × WILD TYPE ♀.

L.	D L.	Bl.	D Bl.
218	188	200	169

B. C., F_1 DICHÆTE BRISTLE LOBE ♀ × WILD TYPE ♂
(DICHÆTE NOT CLASSIFIED).

L.	Bl.	Bl L.	Wild Type.
263	222	47	60

In order to find the approximate location of Bl in the second linkage group F_1 Bristle females from matings of Bristle × black purple curved were back-crossed to black purple curved males. The results (Table II.) show that Bl lies to the right of purple and give the following cross-over values b–p 6.5 per cent; p–Bl 0.18 per cent.; Bl–c 14.6 per cent. and p–c 14.8 per cent. (The standard values are b–p 6 per cent. and p–c 21 per cent. No explanation was found for the discrepancy in the purple-curved region.) As there were only three cross-overs between Bl and p, one of which was also a cross-over between b and p (a rare occurrence in 6.7 units) it seemed advisable to make further tests.

TABLE II.

P_1 BRISTLE ♀ × BLACK PURPLE CURVED ♂.

Bl.	Wild Type.
191	225

B. C., F_1 BRISTLE ♀ × BLACK PURPLE CURVED ♂.

bpc-Bl.	bBl-pc.	bp-Blc.	bBlc-p.	bpBl-c.	bc-Blp.
725 576	33 55	97 122	10 5	1 1	0 1
1301	88	219	15	2	1

A black purple Bristle curved stock was made up by crossing the black purple Bristle female (which was also heterozygous for curved) from Table II. with a black purple curved male. F_1 Bristle females from mating of black purple Bristle curved × wild type raised at 31 degrees C. were back-crossed to black purple curved males for six days only with the results shown in Table III. The recombination percentages are as follows: b–p 14.9 per cent.; p–Bl 1.6 per cent.; Bl–c 23.7 per cent. and p–c 25.3 per cent. which correspond with the values b–p 14.0 per cent. and p–c 26.7 per cent. found by Plough ('17) for the same temperature.

TABLE III.

P_1. BLACK PURPLE BRISTLE CURVED ♀ × WILD TYPE ♂ AT 31° C.
F_1 Bristle ♀ × black purple curved ♂ 6 days only.

bpBlc-Wild Type.	bpBl-c.	bpBl-c.	bc-pBl.	bp-Blc.
167 198	35 25	40 70	12 13	4 5
365	60	110	25	9

That Bristle is lethal when homozygous was indicated when Bristle males and females (from Table I.) were inbred. Such matings gave approximately 2/3 Bristle and 1/3 wild type offspring (Table IV.). Further demonstration was obtained as follows: A black Bristle curved male (from Table II.) was mated to a wild type female and F_1 males and females were inbred. The eight curved and one black offspring out of 145 represent cross-overs and show that homozygous Bristle is not viable. This is further shown by the inbreeding of F_1 Bristle males and females from a cross of Bristle purple (from Table II.) with wild type. No purple offspring appear because purple is so closely linked to Bristle. Bristle does not represent a deficiency toward the purple region at least. A balanced lethal stock with Bristle and Lobe linked, balanced against Curly has been made up and should prove of some value in quickly locating genes in the second linkage group.

Table IV.

Bristle ♀ × Bristle ♂ (from Table I.).

Bl.	Wild Type.
416	213

P_1, Black Bristle Curved ♂ × Wild Type ♀.
F_1 Bristle ♂ and ♀ inbred.

Bl.	Wild Type.	Blc.	bBl.
74	62	8	1

P_1 Bristle Purple ♀ × Wild Type ♂.
F_1 Bristle ♂ and ♀ inbred.

Bl.	Wild Type.
74	56

Summary.

1. A new bristle form in *Drosophila melanogaster* has been found and named Bristle, Bl.
2. The mutant gene is a dominant, lethal when homozygous.
3. The locus of Bl lies 0.18 per cent. to the right of purple at approximately 54.8 in the second linkage group.
4. A stock of Bristle Lobe balanced against Curly has been made up and is available for use.

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EXPERIMENTAL LOCALIZATION OF NEW AXES IN CORYMORPHA WITHOUT OBLITERATION OF THE ORIGINAL POLARITY.

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The various lines of evidence demonstrating the existence of physiological gradients in *Corymorpha* have been considered in earlier papers (Child and Hyman, '26, Child, '26*a*, '26*b*). In the last of these papers it was shown that the differential resulting from contact of one end of a stem piece with the bottom and free exposure of the other may determine the one as basal, the other as apical, irrespective of the original polarity. In accordance with this fact it was shown that in pieces undergoing reconstitution on the bottom the bipolar frequency is lower and the unipolar frequency higher than in those supported on loose cotton near the surface of the water so that the ends are more nearly equally exposed. In other papers it was shown that pieces after subjection to various inhibiting agents may develop new polarities and symmetries quite independent of the original axes and of the cut ends (Child, '27*a, b*). Apparently the inhibiting agents decrease or obliterate the original polarity and symmetry and the localizing influence of the cut ends and under these conditions the differential of position becomes more effective in localizing apical ends on the free surface and basal ends on the surface in contact or near the bottom.

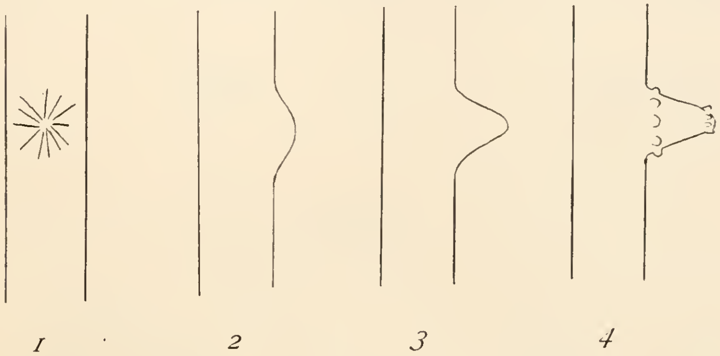
The present paper is concerned with some further experiments on the determination of new polarities. In these experiments the new axes are localized as centers of high metabolic activity and growth without obliterating the original polarity.

Experimental.

No indication of development of new hydranths by budding has been observed in *Corymorpha*. Among thousands of individuals collected, only a single case of more than one hydranth on one stem has been observed. In this case the stem was single over most of its length, but divided into two distally and each of these bore a hydranth. The two hydranths were equal in size and it was not possible to distinguish one as terminal, the other as lateral. Another individual collected possessed a lateral stem outgrowth some ten mm. in length but without hydranth or base at its tip. A third individual possessed two manubria. These are the only cases of axial multiplication found thus far in the collected material. Considering the high frequency of such multiplication under experimental conditions (Child, '27 *a, b*) it seems remarkable that it does not occur more frequently in nature.

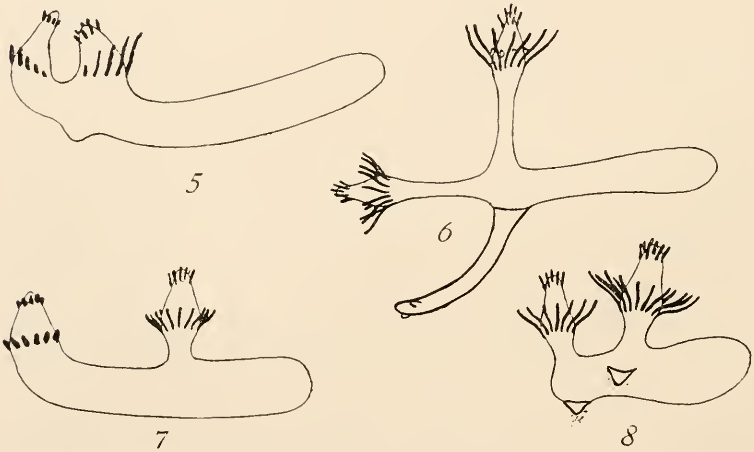
A simple lateral cut with smooth edges, extending a third or even half way through the stem closes within an hour or two under normal conditions and no new apical end or other outgrowth results from it. An earlier experiment on pieces of the actinian, *Harenactis,* suggested that a modification of the procedure employed in that case might determine a new polarity and symmetry in lateral stem regions of *Corymorpha.* In the case of *Harenactis* it was found that when mesenteries and muscles were much injured or in large part removed the shorter transverse pieces contracted in such a way as to bring distal and proximal cut edges of the body wall together and union took place between these edges about the whole circumference, giving rise to ' rings ' (Child, '09*b*). It was found further that in places where the union between the cut edges was smooth and without much new tissue no outgrowths developed along the line of union, while in places where more new tissue developed groups of tentacles appeared. This result led to the further experiment of mutilating opposite regions of the two cut edges by means of numerous small cuts close together and vertical to the edge. When these two mutilated regions came together they could not unite smoothly and extensive growth of new tissue took place before healing was complete. From this new tissue there gradually developed in some cases a new normal individual (Child, '10, Figs. 5, 6). The new apical region appeared only after complete closure of the wound by new tissue. This new tissue gradually bulged outward because of the internal water pressure, continued to grow and finally developed as a new polar axis. The radial symmetry accompanying this new polarity seemed to be primarily merely an expression of the likeness of all radii in a plane vertical to the polar axis.

In the hope that it might be possible to determine a new polarity from the lateral stem region of *Corymorpha* in a manner somewhat similar to that employed in *Harenactis* the stems were cut as follows: with small, fine-pointed scissors cuts one to two mm. in length, radiating from a center, were made as indicated in Fig. 1, the purpose of the operation being merely to localize a region of active cells. In all cases the original hydranth was removed in order that its dominance might not interfere with the development of a lateral hydranth and in some of the earlier series the new hydranths which developed at one or both ends of the piece were also removed in early stages for the same reason, but this was found to be unnecessary. Closure of this wound was slower than in case of a simple cut, but was usually complete in twelve to twenty-four hours. In the successful operations the region began to bulge soon after closure (Fig. 2) and soon became a definite rounded outgrowth which underwent elongation (Fig. 3) and after two or three days attained the form of a hydranth with early stages of tentacles (Fig. 4).



FIGS. 1–4. Development of hydranth from lateral region of growth determined by radiating cuts as shown in Fig. 1.

Figures 5–18 show characteristic results of this operation. In all figures the region of stem covered with perisarc is indicated by heavier outline than other regions and the perisarcal accumulation at the basal end is indicated by dotting. Fig. 5 is a case of new lateral polarity in a piece some twenty-five mm. in length from the middle of the naked region of a 70–80 mm. animal at a stage four days after operation. The apical hydranth of the piece is the second one developed, the first having been removed two days after section. The apical and the lateral hydranth are so near together that they mutually inhibit tentacle development on the sides facing each other and so have acquired a dorsoventrality with respect to each other. The side of the stem opposite the lateral hydranth shows an outgrowth which later becomes a base.



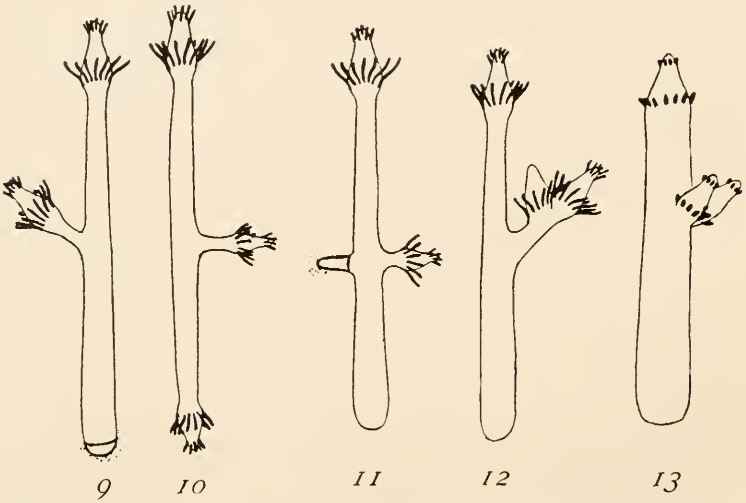
Figs. 5–8. Development of new axes from lateral regions of growth determined by injury. Pieces 25 mm. in length from middle of naked region of animals 70–80 mm. Figures are about twice natural size. Figs. 5 and 6, two stages of a piece developing a complete new axis at right angles to the original polarity. Figs. 7 and 8, two stages of a piece which develops a new basal end in relation to both lateral and apical hydranth.

Fig. 6 represents the condition of the piece three days later. The two hydranths have now developed separate stems and a new base has arisen opposite the lateral hydranth. This development of a new individual from the apical end basipetally following the localization of the new apical end by the injury is an excellent example of apicobasal dominance. The localization of an active region by the injury determined a new hydranth, this determined successive stem regions basipetally until finally a new basal end was determined on the opposite side of the piece, that is, the dominance of the new axis became effective through the stem at right angles to the original polarity. Contact with the substratum may have assisted in determining the lateral stem region as a basal end (Child, '27*a, b, c*), though this region was not found in contact when the piece was observed, but if the new polarity were not concerned in localizing the base it would probably have arisen at or near the proximal end of the piece. At this stage then the piece represents two distinct polarities at right angles to each other. In later stages the form became bipolar-unipolar, the new base becoming the base for both hydranths and the stem region proximal to the lateral polarity gradually undergoing resorption.

Fig. 7 shows another case from the same series at a stage four days after operation. Here also the first apical hydranth was removed and the figure shows the second developing. Fig. 8 shows the same form three days later. Lateral stem regions are developing into a new basal end in relation to each hydranth. In this case as in the preceding, contact may perhaps have been concerned in producing conditions favorable to base development from the side of the piece, but the localization of the two bases in relation to the hydranths indicates that the more distal regions of each axis were to some extent concerned in localizing the bases. In this case also the region of the stem proximal to the lateral hydranth was gradually resorbed and the form became biapical and bibasal. The two individuals would probably have separated completely like most other double forms if they had been kept long enough.

In both of these cases the proximal stem region apparently cannot maintain itself in the presence of the new lateral polarity and is resorbed. The new polarity obliterates the old, probably because the new represents higher levels of metabolism and so is able to grow at the expense of the older stem regions. Such growth of new axes at the expense of old stem regions has been observed frequently in other experiments (Child, '27*a*).

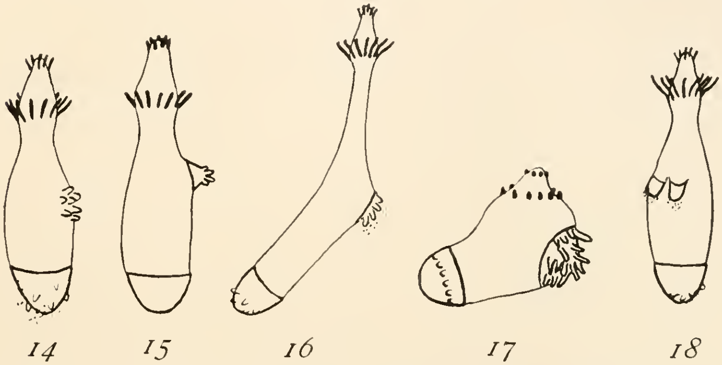
Figs. 9–13 show cases from another series in which the pieces included the whole or almost the whole length of the naked region of 50–60 mm. animals. The figures show stages five days after section. In Figs. 9 and 10 the lateral axes have not developed basal ends, but in Fig. 11 a basal end is developing opposite the lateral hydranth and in Fig. 12 two hydranths have developed from the lateral outgrowth. Fig. 13, a piece of another series from 70–80 mm. animals three days after section is another case of two lateral hydranths.



Figs 9–13. Other cases of lateral hydranth development from region of growth determined by injury. Figs. 9–12 from pieces including the whole naked region of 50–60 mm. animals. Fig. 13 from piece including whole naked region of 70–80 mm. animal. The figures are slightly above natural size.

This lateral operation has been performed on thirty-five stem pieces and of these fifteen, or forty-three per cent. have given rise under standard conditions to new lateral axes consisting of at least a hydranth and more or less stem. Twenty pieces, fifty-seven per cent., healed without giving rise to new axes. Among the new lateral axes three, *i.e.*, nine per cent. of the total, became complete by the development of a basal end from the lateral stem region opposite the lateral hydranth.

It has been shown in earlier papers (Child, '26*b*, '27*a*, *b*), that either cut end or both, or any other region of a piece may develop as a basal end under inhibiting external conditions. In the light of these results it is to be expected that regions of lateral injury may also be made to develop as basal structures under inhibiting conditions. Up to the present, however, only one experiment of this sort under inhibiting conditions has been carried out, but its results are conclusive. Of the twenty pieces each including the naked and half the perisarcal region, which were used in this experiment fourteen, seventy per cent. gave rise to basal structures in the region of lateral injury and one, five per cent., to a hydranth, while five, twenty-five per cent., healed without outgrowth. Figs. 14–18 show characteristic cases from this series four days after operation. In Fig. 14 the region of injury has developed merely a few holdfast or stolon buds, while in Figs. 15 and 16 stolon buds are present at the tip of a general basal outgrowth. In the case of Fig. 17 the stolon buds in the region of injury are more numerous and attain greater length than in any other case. In Fig. 18 the region of injury has given rise to two basal outgrowths. These cases are sufficient to demonstrate that the lateral injury may develop as a basal as well as an apical end.



FIGS. 14–18. Cases of development under inhibiting conditions of basal structures from lateral growing region determined by injury. Pieces include whole naked and half perisarcal region of 50 mm. animals. Figures are about twice natural size.

DISCUSSION.

The experiments described above show that it is possible to localize new polarities in *Corymorpha* stems even without previously obliterating or decreasing the original polarity by means of inhibiting agents. It is important to note that these new polarities resulting from lateral injury are not similar to the lateral partial discs which result from a transverse cut part way through the body in *Cerianthus* (Loeb, '91, Child, '05, '08) and *Harenactis* (Child, '09*a*). In those forms the opening remains because the cut edges of body wall and oesophagus unite and the new partial disc develops entirely on the proximal side of the cut, just as a disc develops on any distal cut end of a piece. In the case of *Corymorpha* the wound closes completely in the course of a few hours and it is only through the continued growth of the region after closure of the wound that the new axis is determined. If the injury does not initiate such growth no new axis develops.

These new lateral polarities are essentially induced buds and like other buds they give us important evidence concerning the origin and nature of new axes. If we observe, without theoretical prejudice, what happens in such a process, we see that the new axis originates as a local region of growth and becomes visible as an outgrowth of the body wall (Fig. 2) because the growth activity is evidently greatest in its middle region and decreases peripherally in all directions. The early rounded outgrowth undergoes elongation and the more active middle region necessarily becomes its tip (Fig. 3), in other words, the region of growth has now become a physiological axis characterized by a gradient in activity decreasing from the tip basipetally. There can be no doubt that when such a gradient is once determined in a particular kind of protoplasm the constitution of the protoplasm will play the chief part in determining its steepness, its length and the changes which it undergoes during development. If we admit this, it follows that however the gradient is determined its definitive form will be the same in a protoplasm of a certain constitution, consequently a gradient such as the one under consideration, determined by a local injury will determine the same course of development, *i.e.*, the same kind of an axis as the gradient in embryonic development, if the condition of the cells in the region of active growth is similar to that of embryonic cells of *Cormorpha*. If we take the facts as they stand it seems that there is no adequate reason to regard a polar axis in its simplest form as anything more than such a gradient as this. Experiment shows that when such a gradient is determined a new axis is determined and when the gradient is obliterated the polarity is obliterated, so far as can be determined.

It has been shown for many forms, both plant and animal, that buds originate as gradients of this sort, resulting merely from the localization of an active region which is not sharply marked off from its surroundings but shows a gradient of decreasing activity from a central region toward the periphery. In consequence of differential growth, which itself results from the existence of this gradient, the radial gradient becomes an apicobasal gradient and a polar axis. There is no evidence to indicate that a polar axis is primarily anything more than such a dynamic differential with its structural protoplasmic correlates, or that differentiation along an axis requires anything more for its initiation than the quantitative differences at different levels of such a gradient.

The development of basal instead of apical structures from a lateral injury under inhibiting conditions is in complete agreement with the results of other experiments. It has been shown that the basal region of *Corymorpha* represents a secondary gradient which originates at the low end of the primary gradient (Child and Hyman, '26; Child, '26*a*). The high end of this secondary gradient, so long as it persists, is the basal tip and the slender modified stolons which constitute the holdfasts develop as lateral buds along this secondary gradient. These stolons show extremely rapid growth, but they originate only in regions of relatively low activity. When the activity of the region of lateral injury is decreased to a certain degree by inhibiting factors, the conditions must become more or less similar to those existing at the lower end of the primary gradient, and the lateral injury, like the lower end of the primary gradient, develops as a basal region. Whether a single basal outgrowth bearing stolon buds, or merely the stolon buds appear probably depends on various factors, *e.g.,* the degree of inhibition, the presence or absence of a definite growth region, etc. If a single general basal outgrowth arises the further development of the basal gradient and basal region follows in the same way as the development of the hydranth-stem gradient and region. Even if the central growth area resulting from the injury is not sufficiently well defined to determine a single general basal outgrowth, new stolon buds may be determined in relation to the entodermal canals or parts of canals in the injured region. Since the canals have been mutilated by the injury the arrangement of the stolon buds is likely to be irregular, as in Figs. 14–18.

Development of two apical or basal ends from the region of injury is undoubtedly a result of determination by the injury of two regions of activity instead of one. Duplication of this kind has been very widely observed in many forms as a result of splitting or otherwise dividing a growing region into two.

One of the most interesting results of these experiments is the determination by the more distal levels of the new axis of a basal region on the opposite side of the stem where there is no injury (Figs. 5, 6, 8, 12). It is evident that the development of the distal region of the new axis has in some way altered conditions in the region of the stem which gives rise to the base, but it has been shown that contact or nearness to the bottom and the action of various inhibiting agents may alter conditions in the same direction in regions of the stem (Child, '26*b*, '27*a*, *b*). This being the case there is no good reason for supposing that the changes which initiate the development of the basal end are anything more than quantitative changes in physiological condition determined by the presence of the new gradient. In the case of Fig. 8 in which the apical hydranth takes up a more or less lateral position because of the position of the piece, it, as well as the lateral hydranth, develops a new basal end, perhaps with the assistance of the conditions resulting from contact of the region concerned with the bottom.

The new lateral axis develops the characteristic radial symmetry, except in cases such as Fig. 5, in which the differential resulting from proximity of the other hydranth determines mutual and opposed dorsoventrality. If we examine the facts, again without theoretical prejudice, it appears that the radial symmetry of the axis is primarily nothing more than likeness of all radii in a plane perpendicular to the polar axis. The primary growing region determined by the injury is more or less radially symmetrical because its activity decreases radially from a center and as it becomes a definite outgrowth (Figs. 2, 3) its radial symmetry appears to result from this radial differential and from the fact that a surface-interior differential exists at all points. With the localization of tentacles certain radii become different from others. The factors concerned in tentacle localization have been but little investigated, but it is difficult to believe that the localization begins independently of external factors of some sort which determine where the first tentacle or tentacles shall appear. In the ordinary course of reconstitution the entodermal canals are important factors in localizing the new tentacles, as Torrey has shown (Torrey, '10). Factors concerned in tentacle localization in the lateral polarities have not been studied, but they will probably be found in the relations of the growing region to the rest of the stem. Study of *Hydra* and various hydroids indicates that localization of a single tentacle is sufficient to initiate the orderly development of others. Torrey's study of the order of appearance of tentacles in the embryonic development of *Corymorpha* is interesting in this connection as indicating that different localizing factors are concerned in different individuals for he finds that the process does not follow a uniform course (Torrey, '07). Apparently each region of growth, whether tentacle or other organ, dominates a certain area so that a similar organ cannot develop within that area. When a particular tentacle is localized, for example, another can develop only outside its range of dominance. Any part of the circumference in the tentacle forming region is undoubtedly capable of giving rise to a tentacle, but the actual localization in a particular case must depend on the factors concerned. That the outgrowth which becomes a polar axis with a radial symmetry can localize its own tentacles independently of any of the external differentials to which it is exposed is at least highly improbable and seems to require the action of some non-mechanistic ordering factor. If these observations and suggestions are correct, radial symmetry in these new axes has its origin in the primary likeness of radii at any particular level and in the difference between surface and interior which is present in some form in all organisms. The later localization of a series of similar organs in radial arrangement seems to demand the action of some differential external to the parts concerned.

Summary.

1. Buds have never been seen to arise from lateral stem regions in *Corymorpha* and a simple transverse cut into the side of the stem closes rapidly without development of a bud or other outgrowth, unless it extends almost through the stem.

2. A region of injury produced on the side of the stem by short cuts radiating from a center closes less rapidly than the simple transverse cut and in many cases gives rise after closure to a rounded outgrowth which becomes conical and develops into a new hydranth. This hydranth develops a stem at the expense of the old stem and in some cases the new axis determines a new basal end on the opposite side of the piece, thus completing a polarity at right angles to the original axis. Occasionally two hydranths instead of one are localized by the injury.

3. The experimental data indicate that the new polarity and symmetry are the necessary consequence of the localization of a center of cellular activity. The radial gradient of decreasing activity from the center peripherally becomes, as growth proceeds, the axial gradient and the radial symmetry is primarily merely a similarity of all radii vertical to the polar axis at any level.

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